THE RELATIONSHIP BETWEEN PERIODONTAL CONDITION AND CERTAIN DIETARY FACTORS[1]

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INTRODUCTION

Disease of the investing tissues of the teeth is prevalent in adults and is a significant factor in the loss of human teeth. Clinical experience was corroborated by the statistics of Brekhus (1929) which showed that although caries and pulp disease were the predominant conditions responsible for the loss of human teeth before the age of 30, periodontal disease (periodontoclasia, periodontitis) became the most common factor beginning at the age of 20 and increasing consistently in importance up to the age of 70, which was the age limit shown in the record.

Various reasons for this wide-spread occurrence of periodontal disease have been advanced. Improper oral hygiene, local mechanical factors, or systemic influences, including various nutritive factors, have been considered dominant in the production of the disease.

Reports show that diet can influence the structure of dental investing tissues. There is also increasing evidence of disease entities which are due to a sub-optimal intake of certain dietary factors which may not be low enough to produce the generally recognized or accepted symptoms of deficiency diseases. The presence of typical periodontitis after maturity suggests the possibility that this condition may be a symptom of sub-optimal diet. Therefore, it seemed desirable to study statistically the relationship between the intake of certain dietary factors and the periodontal condition of patients.

HISTORICAL

Surveys like that of Simmonds (1938) have shown that only a few patients who seek dental services are, regardless of economic

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status, on an optimal diet, judged by generally accepted dietary standards. Confusion as to the interpretation of the rôle of diet in periodontal disease arises because deficiencies are commonly multiple and are generally only partial.

Investigations by Sherman and Booher (1930), Yeager and Winters (1935), Bauer, Aub, and Albright (1929), and Templin and Steenbock (1933) demonstrate that bone deficiencies can be produced by calcium deprivation. Becks and Weber (1931) and Toverud (1932) found changes of the alveolar bone of animals fed on low-calcium diets to be more extensive than changes in the other bones. Yudkin, Farquhar, and Wakeman (1934), and Arnim, Clarke, Anderson and Smith (1936) realimented rats which had been maintained on low-salt diets, by administering adequate diets, thereby producing repair of bone including alveolar bone. Extraction of maxillary molars caused changes in the jaw bones of both control and low-salt rats, but the changes were exaggerated in the rats maintained on low-salt diets (Anderson, Smith, Arnim and Orten, 1936).

Vitamins also influence periodontal tissues. The experiments of Mellanby (1930), Wolbach and Howe (1931), and King (1936) suggest the importance of vitamins A and D. Howe (1922), and Boyle, Bessey, and Wolbach (1937) described various periodontal tissue changes in guinea pigs on a scorbutic diet. Freeman and Glass (1938) found no correlation between blood ascorbic acid levels and incidence of bleeding gums and gingivitis in a group of human adults, composed of psycotic patients and the hospital staff. Evidence pointing to the need for consideration of vitamin C as a factor in periodontal changes under special dietary regimes and of the value of small amounts of dietary vitamin C in providing tissue protection has been presented by Weisberger, Young and Morse (1938). The fact that, although Jones and Simonton (1928), Mellanby (1930), Becks and Weber (1931), King (1936) and others have shown that dogs are susceptible to periodontal disease, according to Svirbely and Kendall (1936) and Vars and Pfiffner (1934) dogs can be kept free from scurvy on a vitamin C-free diet, suggests that some other factor or factors are commonly of greater importance.

The influence of the acid-base balance of the diet is considered by some to be pertinent. Sendroy (1939) in a review of the literature on the subject concluded that "the effect of diet tends to be minimized as accumulating evidence continues to show that no appreciable alteration of the acid-base balance by diets of an acidifying or alkalinizing nature is possible." Sherman (1937) stated that there were some indications favoring a balanced intake of acid-forming and base-forming elements, but he added that it was still an open question whether the acid-base balance of the diet is of practical importance.

EXPERIMENTAL METHOD

Collection of Data. Patients presenting themselves for prophylactic or periodontal treatment at the school dental clinic were requested to keep a list of all food eaten in 1 week (7 days), stating approximate amounts of each serving. Unless otherwise specified, it was considered that servings of foods were average in size. All foods and beverages consumed between meals were to be included. Blank paper was used for keeping the record since it was felt that printed forms might suggest foods which were not representative of the usual diet. To insure relative accuracy, the diet record was checked over with each patient. To these records were added those of private office patients (16).

There has never been an exact compilation of the economic status of dental school clinic patients. In the present study, without resorting to an accurate check, many of the patients were found to be faculty members or students; others apparently belonged to the middle class while some obviously came from the lower income groups. The clinic is situated on the main University campus and recognizes no limitation for admission, patients not being drawn exclusively from the lowest income groups as the term "clinic patient" might suggest.

Information was included as to sex, age, height, weight, number of teeth present, previous periodontal treatment, diet changes during the past year, vitamin preparations used, and medical history. Periodontal tissue changes were recorded on a standard chart and were checked clinically by 1 of 3 experienced members of the staff including the author. Radiographs were required in every case, and were examined by the author. The cases were classified by a study of the relative bone loss and notations of gingivitis. No attempt was made to differentiate or group cases on the basis of various forms of gingivitis; therefore it did not seem necessary for 1 operator to examine all the patients. Simple recession of the gingival tissue was not considered pathological if no other reactions of this tissue were observed.

The broad classification of degree of involvement was based primarily upon the bone changes, as shown by radiographs, coupled with the presence of gingivitis. Bone criteria alone are undesirable not only because the disease involves both hard and soft tissues, but also because arrested cases which would show no gingival lesions might be included in the diseased groups. No attempt was made to eliminate cases which had received previous treatment if, when they came to the clinic, they showed both bone loss and gingivitis. All cases were classified before evaluating the diets.

The following grouping was used:

Class A.....no apparent gingivitis or bone loss
B.....general gingivitis with slight bone loss
C.....general gingivitis with bone loss up to one-third (moderately advanced)
D.....general gingivitis with bone loss one-third to one-half (advanced)
E.....general gingivitis with bone loss more than one-half (very advanced)

Patients having 20 teeth or more present were arbitrarily selected for observation, because with greater numbers of teeth missing it was often impossible to ascertain reasons for previous loss. Selection of patients having at least 20 teeth would permit avoidance, too, of some of the conflicting symptoms often produced by the mechanical influences brought about when many teeth are missing. To minimize contributing mechanical factors it would have been better to select patients retaining all their teeth except the third molars; but such cases over a range of ages are too infrequent for statistical study.

All records lacking adequate dietary or other information were omitted from the evaluation as well as those of patients who were under medical dietary supervision. The study was planned to include only those patients who were exercising *free choice of diet*. However, there was no sure way of ascertaining that the week chosen for keeping the diet record would provide a representative sampling of the dietary habits of the patient. Yet some check was provided by the question, "Has there been any change in diet during the past year?"

Dietaries taken at the clinic were obtained during the months of October to June, and those of office patients during the summer months and early fall. The collection period began in January, 1936, and ended in April, 1938.

Diet records of a group of 96 periodontic patients composed predominantly of private practice cases but with a few from the clinic of the Harvard Dental School reported by Tishler (1929) were obtained from him and subjected to the same type of evaluation as the experimental group. Classification of the degree of periodontal involvement necessarily had to be based upon Tishler's records. Therefore, although these cases were arranged in the proper order according to involvement, the moderately advanced cases, for example, might not be exactly comparable to the moderately advanced cases of the experimental group.

Dietary Evaluation. The 7 day diet records of each patient were estimated and averaged per day for content of protein, animal protein, calcium, phosphorus, and iron in grams; vitamins A and C in Sherman units, and acid-base excess in ccs. of normal acid or alkali. Those of the female experimental group were also estimated as to milk content. Waller's tables (1937) were used as reference for protein, calcium, phosphorus, iron, and acid-base excess. Daniel and Munsell's (1937) tables were used for vitamins A and C; the vitamin C values for the cooked and raw foods as therein reported being used without following the procedure of the Bureau of Home Economics of estimating all vitamin C on the uncooked food basis.

This method of evaluation was considered feasible because Bauer and Aub (1929), Bassett, Elden, and McCann (1931) and Ohlson and Daum (1935) had found what they considered as reasonably satisfactory agreement between calculated and determined food values. Bassett, Elden, and McCann showed that in comparing the results of analyzing

an entire diet for a day with those secured by calculating the total mineral intake from Sherman's tables, the values actually found for calcium were consistently lower (15.5 per cent) than the anticipated calculated values. These results confirmed the work of Bauer and Aub. The consistent lower value for estimated calcium would not invalidate statistical findings based on a grouping of cases from a continuous scale. Discrepancies discovered in the case of nitrogen, phosphorus, potassium, magnesium, or iron were smaller and within the limits of accuracy of the procedure as a whole. The same diet menus weighed out and analyzed in different years and different seasons showed surprisingly close agreement, although these authors suggested the possibility that geographic areas from which food supplies may come could conceivably vary the magnitude of difference.

Statistical Method. Data cards were compiled for each case and histograms were prepared for each dietary factor for each male and female group separately. From these the data cards were coded and correlation tables showing bivariate distribution between each dietary factor and degree of disease were made.

Since the dependent variable, the degree of periodontal disease, did not lend itself to quantitative measurement, the correlation ratio could not be used. Therefore the coefficient of mean square contingency (C) was the method used for further statistical computation. The measurement of frequency discordance or the "Chi-squared" (X^2) value was first computed; and the probability (P) of the X^2 value being exceeded solely through sampling errors was determined from Elderton's table (Pearson, 1924). If the probability (P) is less than .05 there is a high probability favoring the deduction that there is association between two variables, their occurrence together not being due only to chance. When P was less than .05, the coefficient of mean square contingency (C) was computed.

The same procedure was followed for each factor as illustrated by the calcium statistics for the female experimental group presented in *figs. 3, 4, and 5.* As the female experimental group was the largest, 135 cases, it was chosen for computation of the partial and multiple correlations substituting the coefficient of contingency for the correlation coefficient in the formula. The sign, (plus or minus), was determined by examination of the difference between observed and calculated occurrence in each cell. This procedure was necessary since the contingency coefficient has no sign.

Formulae: Formulae and methods illustrated by Treloar (1935) were used. In some instances alternative techniques have been described by Treloar. In such cases the following formulae were chosen as best fitting the data.

"Chi-squared," $X^2 = \sum^{m} [(o-c)^2/c]$ where m is the classes, o the observed frequency, c the calculated frequency. In this formula the values of $[(o-c)^2/c]$ are totaled for the entire series of m classes.

Coefficient of Mean Square Contingency,

$$C = \sqrt{X^2/(N + X^2)}$$

where N is the number of cases.

Partial Correlation (3 variables),

$$r_{cd.p} = \frac{r_{cd} - r_{cp}\, r_{dp}}{\sqrt{(1 - r_{cp}^2)(1 - r_{dp}^2)}}$$

where c is calcium, d is periodontal condition, and p is phosphorus. Proper substitutions were made for each computation for the various factors studied.

Multiple Correlation, (3 variables),

$$r_{d.cp} = \sqrt{\frac{r_{cd}^2 + r_{dp}^2 - 2r_{dc}r_{dp}r_{cp}}{1 - r_{cp}^2}}$$

where d is the periodontal condition, c is calcium, and p is phosphorus. Again, proper substitutions were made when computing the other factors.

DATA

The cases studied were divided into 4 groups and designated as male and female Tishler and male and female experimental groups as shown in *fig. 1.* The distribution of cases among the 5 classes of periodontal condition is represented by histograms. Seventy-three of the 96 Tishler patients (76 per cent) are found in the advanced (D) or very advanced (E) classes, there are no healthy periodontal cases, and only 2 beginning (B) cases. Of the 184 experimental cases, 37 have no recognized symptoms (A), 30 display slight involvement or beginning periodontitis (B), 68 are found in the moderately advanced (C) class, and 49, or 27 per cent, in the advanced (D) and very advanced (E) class. Nearly a third of the Tishler cases had very advanced lesions, while only 10 out of 184 experimental cases were found in this class. Perhaps there was a difference in interpreting the meaning of the terms; or there may have been an actual difference in the degree of involvement; or the procedure of handling patients at the dental clinic may have produced the difference. All patients presenting themselves for treatment at this clinic are required to pass through the diagnosis department before being referred to the various departments. By this process the very advanced cases may have been eliminated from this investigation because of direct reference to the surgery department for extraction of the affected teeth. The prophylaxis or healthy cases (A) were included in the experimental group to provide a continuous scale of tissue differences. No doubt it would have been better if all patients presenting themselves at the diagnosis department could have been included in the study. Such procedure would have lessened the chance for bias in the sample. In setting up the experiment, it was planned to compare the differences in intake of the various factors in the healthy and diseased cases. But it was soon discovered that even a "prophylaxis" clinic could not supply a sufficient number of cases with healthy periodontal tissues, so this procedure could not be carried out.

The correlation tables in *fig. 2* present the distribution of patients according to degree of periodontal involvement and age. The bulk of the patients ranged in age from 25 to 55 years. It is noteworthy that the healthy cases are distributed over the entire age range, and are not confined to the group under 25 years of age.

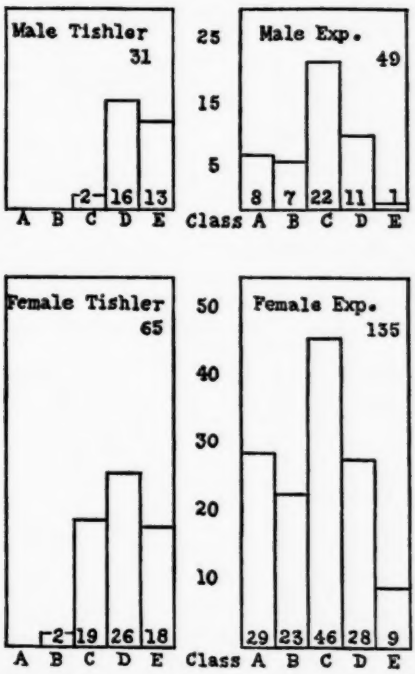

FIG. 1. Distribution of patients according to degree of periodontal involvement

Key: Class A—healthy, Class B—beginning disease, Class C—moderately advanced, Class D—advanced, Class E—very advanced.

The estimated daily calcium intake extended over the wide range of 0.12 gram to 1.65 gram shown in the histograms of *fig. 3*. The intake of the Tishler groups ranged from 0.12 gram to 0.88 gram, while the experimental groups, which included healthy periodontal cases, had the greater range of 0.14 gram to 1.65 grams of calcium per day.

Fig. 4 carried the data a step further by correlation tables which

show the joint occurrence of degree of involvement and calcium intake. All but 5 of the 96 Tishler cases had an estimated intake of less than 0.7 gram of calcium. Thirty-seven of the 184 experimental

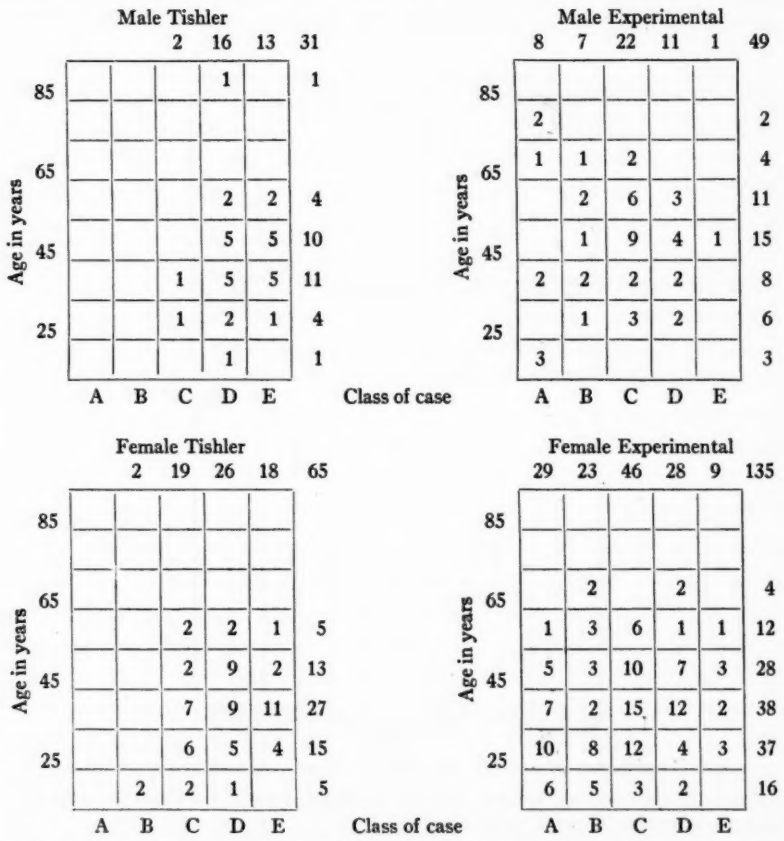

FIG. 2. Correlation tables showing degree of periodontal involvement according to age
Key: Class A—healthy, Class B—beginning disease, Class C—moderately advanced, Class D—advanced, Class E—very advanced.

cases ingested more than this amount, yet only 17 of the healthy (A) patients were found with this commonly stated "standard" intake which allows a margin of 50 per cent above the requirement of 0.45 gram for a 70 kilogram person. Still, 28 of the 37 class A patients

consumed 0.55 gram or more, the amount considered by Leitch (1937) after statistical analysis and study of all published calcium balance experiments, to be the "maintenance requirement" for human adults between 50 to 65 kilograms in weight regardless of sex. No class A (healthy) cases had less than 0.25 gram, but 9 were under 0.55 gram. Examination of the swarm in the correlation tables of *fig. 4* suggests a negative correlation between periodontal disease and calcium intake.

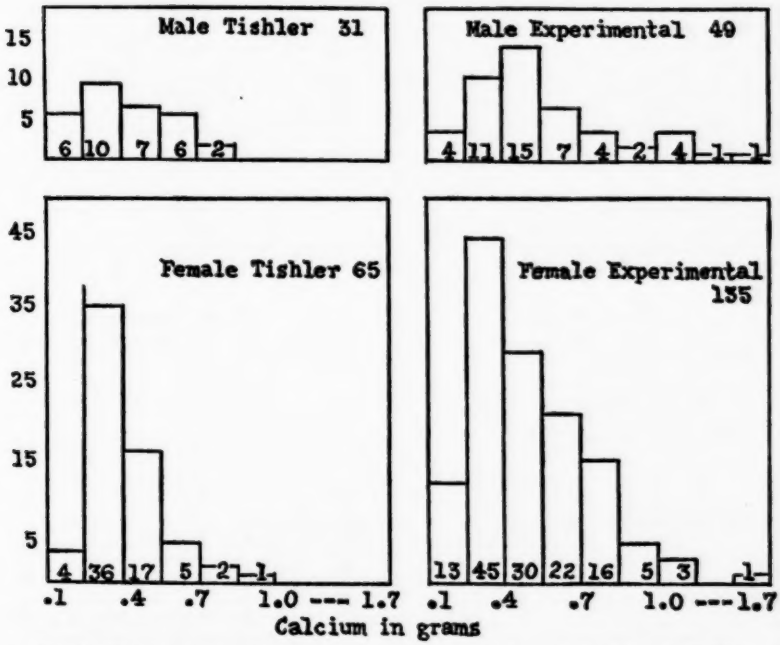

FIG. 3. Histograms showing estimated calcium intake for male and female Tishler and experimental groups.

Fig. 5 shows the contribution to "Chi-squared" (X^2) in each cell of the table for calcium intake and periodontal condition for the experimental group of 135 females. Since X^2 is 48.45 for 12 degrees of freedom, the probability (P) of this value being exceeded solely through sampling errors is .00001. This value is so small that there can be no doubt of the significance of correlation between calcium intake and periodontal condition. Since the probability does not

indicate the intensity of the correlation, the coefficient of mean square contingency was computed, and found to have the value 0.51. Ex-

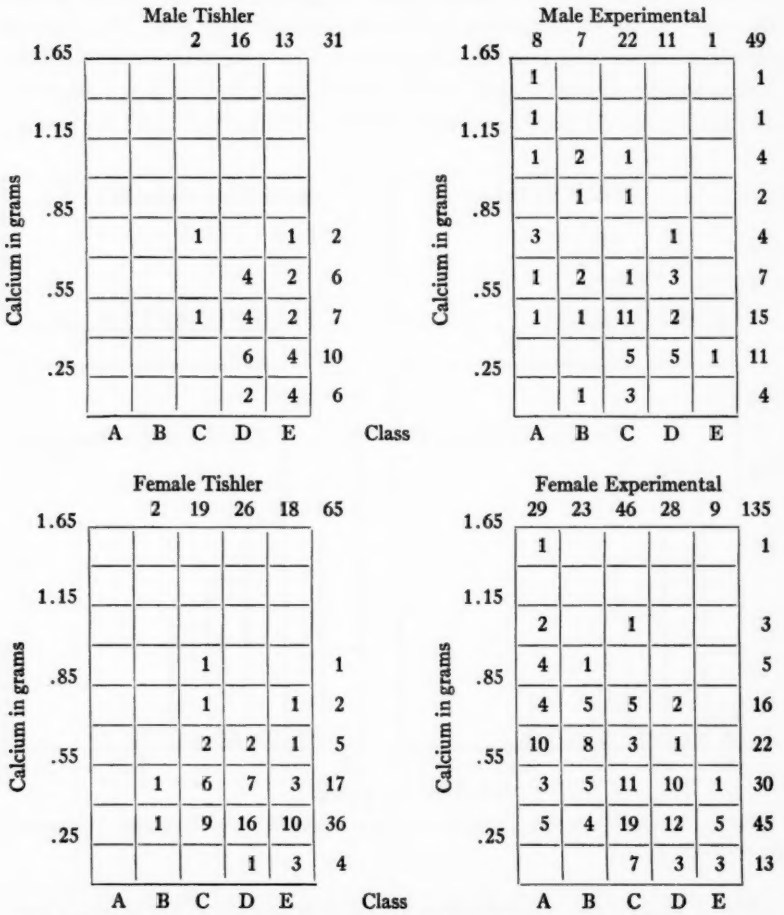

FIG. 4. Correlation tables showing degree of periodontal involvement and calcium intake
Key: Class A—healthy, Class B—beginning disease, Class C—moderately advanced, Class D—advanced, Class E—very advanced.

amination of the difference between observed and calculated occurrence in each cell of the contingency table shows this to be a negative correlation between calcium intake and periodontal disease.

Calcium in grams	A	B	C	D	E	
	29	23	46	28	9	135
	11 5.3650 +5.6350 5.9186	6 4.2550 +1.7450 .7156	6 8.5100 −2.5100 .7403	2 5.1800 −3.1800 1.9517	0 1.6650 −1.6650 1.6650	25
.70						
	10 4.7212 +5.2788 5.9123	8 3.7444 +4.2556 4.8366	3 7.4888 −4.4888 2.6906	1 4.5584 −3.5584 2.7777	0 1.4652 −1.4652 1.4652	22
.55						
	3 6.4380 −3.4380 1.8359	5 5.1060 −.1060 .0022	11 10.2120 +.7880 .0508	10 6.2160 +3.7840 2.3035	1 1.9980 −.9980 .4985	30
.40						
	5 12.4468 −7.4468 4.4553	4 9.8716 −5.8716 3.4924	26 19.7432 +6.2568 1.9828	15 12.0176 +2.9824 .7401	8 3.8628 +4.1372 4.4311	58
	A	B	C	D	E	

Periodontal Condition

Cell Key:		Data:	
observed frequency	o	X^2	48.25
calculated frequency	c	d/f	12
observed—calculated	(o—c)	P	.00001
contribution to X^2	X^2_o	C	0.5

FIG. 5. Analysis for contingency correlation (C) of calcium and periodontal condition

The coefficient of mean square contingency (C) underestimates the true association. The upper limit of C for the various groupings (m) used in this study is:

m	C
3	.82
4	.87
5	.89

Therefore, as perfect C is not unity (1), the contingency coefficient really measures the minimum value. The contingency coefficient is a "grade interpretation," of value to one decimal place only. Use of a measure of association which underestimates the situation is

preferable in cases where measurement of the variables cannot be exact. In this study, even the numerical data are estimations.

The essential results found in computing "Chi-squared" (X^2), probability (P) and the contingency coefficient (C) for the various

TABLE I

Showing X^2, degrees of freedom (d/f), P, and C for dietary factors of calcium, phosphorus, protein, animal protein, iron, acid-base excess, vitamin A, vitamin C, milk, and age with periodontal condition

GROUP*	NO. OF CASES	CALCIUM				PHOSPHORUS				PROTEIN			
		X^2	d/f	P	C	X^2	d/f	P	C	X^2	d/f	P	C
F Exp.	135	48.60	12	.00001	0.51	35.30	16	.009	0.46	30.06	12	.0027	0.43
M & F Exp.	184	62.80	16	10^{-6}	0.50	42.04	16	.0012	0.43	24.70	9	.0033	0.34
M & F Exp. (C, D, E cases)	117	5.84	4	.199	—								
M & F Tish.	96	21.39	9	.013	0.43	8.13	9	.50	—				
F Exp. & Tish.	200	93.60	12	10^{-6}	0.56					41.00	9	.0005	0.35

		ANIMAL PROTEIN				IRON				ACID-BASE			
		X^2	d/f	P	C	X^2	d/f	P	C	X^2	d/f	P	C
F. Exp.	135	24.60	12	.017	0.39	16.80	9	.058	0.33	15.20	9	.086	0.32
M & F Exp.	184	11.98	9	.25	—					18.40	9	.03	0.30

		VITAMIN A				VITAMIN C				AGE			
		X^2	d/f	P	C	X^2	d/f	P	C	X^2	d/f	P	C
F. Exp.	135	7.10	6	.31	—	8.00	9	.64	—	12.70	6	.049	0.29
M & F Exp.	184	17.09	12	.149	—	14.60	12	.27	—	31.30	16	.016	0.38
M & F Tish.	96	2.70	6	.84	—	4.60	6	.61	—	10.80	4	.05	0.32

		MILK			
		X^2	d/f	P	C
F. Exp.	135	32.60	12	.002	0.44

* F is female; M is male; Exp. is Experimental; Tish. is Tishler.

dietary factors for the different groupings of the cases studied are collected in Table I. Examination of this table shows that in every instance, computation of P indicated significant association between calcium intake and degree of disease. Results for phosphorus were not so consistent, but data of the experimental groups indicated a

significant correlation for this factor as well as for protein and animal protein. Iron, age, and acid-base excess show border-line probabilities. Vitamin A and vitamin C probabilities are greater than .05, therefore correlation is not significant in either case. The correlation between periodontal disease and milk (including calcium-equivalent in cheese) intake is 0.44, only .07 less than that for calcium which is 0.51.

Table II presents the association data for calcium as related to the other dietary factors. Examination of X^2 and P for these factors in the case of the female experimental group reveals association in every case. It is positive, and C is either 0.7 or 0.6 for phosphorus, protein, and animal protein, and 0.4 for iron and acid-base excess. This indicates that calcium, phosphorus, and protein are all likely

TABLE II

Showing X^2, degrees of freedom (d/f), P, and C, for certain dietary factors and calcium in the female experimental group

FACTORS CORRELATED WITH CALCIUM	X^2	d/f	P	C	SIGN
Phosphorus	128.70	12	10^{-6}	0.70	plus
Protein	75.30	12	10^{-6}	0.60	plus
Animal protein	92.45	12	10^{-6}	0.64	plus
Iron	32.02	12	.00279	0.43	plus
Acid-base Excess	38.99	12	.00007	0.47	plus
Age	15.15	12	.240	—	

to be supplied by the same foods. Also that if calcium is present, the diet tends to be more basic in reaction. The association between calcium intake and age is not significant since P is 0.24. Therefore, in this study, adults of no particular age range were more likely to include high-calcium foods in their diets.

The contingency correlation, partial and multiple correlations obtained for periodontal condition (d) and each of the dietary factors for the experimental group of 135 females are listed in Table III. The coefficient of contingency (C) was substituted for the correlation coefficient (r) in the equations. The correlation between calcium and periodontal condition when phosphorus is held constant is $-.30$ as contrasted to $-.51$ when phosphorus is not limited, indicating the need for phosphorus. Its correlation with periodontal condition is

−.46, a figure not quite so high as that for calcium. The negative correlation between the periodontal condition and calcium, phosphorus, protein, animal protein, iron and acid-base excess indicates that as the ingestion of these factors is lowered, the periodontal condition tends to become more severe. The positive correlation of +.36 between age and periodontal condition indicates progressively greater tissue involvement with increase in age.

Since estimation of calcium content of the diet permitted the best prediction of the periodontal condition, it seemed desirable to determine whether inclusion of other factors might improve prediction.

TABLE III

Showing contingency correlation, partial and multiple correlations between periodontal condition and the dietary factors considered in the experimental group of 135 females

(The contingency coefficient is substituted for the correlation coefficient)

r_{cd} −.51 r_{ep} +.70 r_{ea} +.60	r_{ad} −.43 r_{ed} −.39 r_{dp} −.46	r_{fd} −.33 r_{hd} −.32 r_{ce} +.64	r_{yd} +.36 r_{cy} −.32 r_{ef} +.43	r_{eh} +.47
$r_{cd.p}$ −.30		$r_{ep.d}$ +.61		$r_{pd.e}$ −.17
$r_{cd.a}$ −.35		$r_{ea.d}$ +.49		$r_{ad.e}$ −.13
$r_{cd.e}$ −.37		$r_{ce.d}$ +.56		$r_{ed.e}$ −.10
$r_{cd.f}$ −.44		$r_{ef.d}$ +.32		$r_{fd.e}$ −.14
$r_{cd.h}$ −.43		$r_{eh.d}$ +.33		$r_{hd.e}$ −.11
$r_{cd.y}$ −.45		$r_{cy.d}$ −.17		$r_{yd.e}$ +.44
$r_{d.ep}$ +.53	$r_{d.ea}$ +.53	$r_{d.epy}$ .56	$r_{d.eh}$ +.52	$r_{d.cy}$ +.55

d—periodontal condition, a—protein, h—acid-base excess, c—calcium, e—animal protein, y—age in years, p—phosphorus, f—iron.

The partial and multiple correlations in Table III amply demonstrate that the inclusion of other factors is hardly warranted. For example, the correlation between periodontal condition and calcium is −.51; the prediction from calcium and age together is +.55; and from calcium, phosphorus, and age 0.56. Any change affected only the second decimal place of the correlation coefficient. It must be emphasized that these predictions were computed from food sources of these factors. It is conceivable that when chemical compounds of calcium and/or phosphorus are the source of these elements in the diet, the correlation might be altered. It must be kept in mind that high-calcium foods supply many factors in addition to calcium.

The scatter diagram, *fig. 6*, combining calcium and milk intake (or cheese-calcium equivalent), is a graphic presentation of the primary source of calcium in diets. Only a small amount of calcium was obtained from other foodstuffs, as can be seen by the fact that the dots tend to cluster along a line. The correlation between milk and calcium intake was computed from basic data, without seriation, and found to be 0.95. The mean calcium intake for the females of the experimental group was 0.50 gram; the standard deviation was 0.23 gram. The mean for milk intake was 19 tablespoons, or a little more than 1 cup; and the standard deviation was 13 tablespoons. The mean intake is comparable to the present average consumption of milk in the United States which is only a little over 1 cup per person per day (Dairy Council Digest, 1938). The high correlation between calcium and milk demonstrates further that the greatest amount of the calcium found in the diet is contributed by milk or cheese.

The results of computations of the means, standard deviations, and correlation coefficients for calcium and phosphorus intake, employing seriation of data of the experimental and Tishler groups, are summarized in Table IV. When only those experimental cases which show definite (moderately advanced to very advanced, or C-E) periodontal lesions are compared to the Tishler data, there is no statistically significant difference in the mean calcium intakes of 0.406 and 0.418 gram. The mean calcium intake of the male experimental group was 0.56 gram, the highest of any group. There was no undue proportion of healthy cases in the males, but the total number of males was only 49. In every instance the calcium and phosphorus mean is well below the recommended standard for the 70 kilogram man.

It is interesting to note the proportion of cases falling between certain levels of intake of dietary factors. Such a summary covering the 135 females in the experimental group is presented in Table V.

Assuming that the average weight of an adult women is 56 kilograms, the Sherman standards formulated for a 70 kilogram man have been adjusted, whereupon satisfactory intake of calcium is considered to be 0.56 gram; phosphorus, 1.1 gram; and protein, 55 grams. The iron allowance for women as given by Sherman (1937) is 12 milligrams. Keeping in mind these selected standards, the intake of the experimental group can be studied. It will be seen that approximately

two-thirds of the women received less than 0.55 gram of calcium per day, and only 25 had as much as 0.7 gram. The preponderance of low intakes is particularly significant if estimations of calcium intake are actually 15.5 per cent (Bassett, Elden, and McCann, 1931) higher

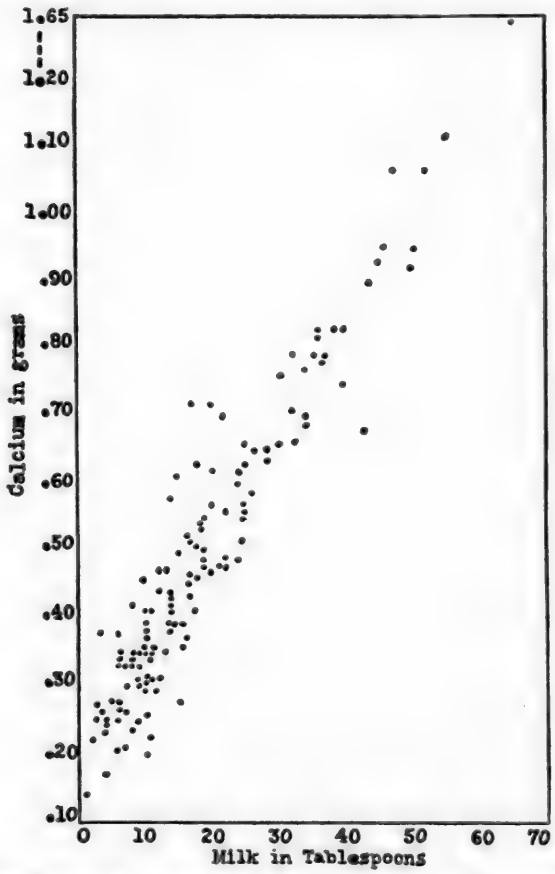

FIG. 6. Scatter diagram for milk and calcium intake of 135 females

than the true intake. If Bassett, Elden, and McCann are correct, even those patients ingesting 0.55 gram of calcium would not be receiving the allowance suggested by Sherman (1937) of 50 per cent of the requirement for a margin of safety.

According to the standards, as stated above, more women displayed phosphorus-deficiency than they did calcium-deficiency, since only 24 were in the range of 1.1 grams or more of phosphorus. About one-half (72) had a protein intake of 55 grams or more, while only 41 ingested 36 grams of animal protein.

TABLE IV

Showing mean, standard deviation (S.D.), and r for calcium and phosphorus in the diet for the various experimental and Tishler groupings

(Employing seriation of data)

GROUP*	N	Ca-MEAN	S.D.	P-MEAN	S.E.	r_{sp}
M & F, Tish. (C, D, E cases)...	96	0.41 g ± .016	0.15 g	0.83 g	0.18 g	0.68
M & F, Exp. (C, D, E cases)..	117	0.42 ± .017	0.18	0.86	0.21	0.77
M & F, Exp.....	184	0.51	0.24	0.93	0.25	0.83
F, Exp.....	135	0.49	0.30	0.88	0.22	0.87
F, Tish.....	65	0.40	0.14	0.80	0.16	0.59
M, Exp.....	49	0.56	0.38	1.05	0.28	0.85

* F is female; M is male; Exp. is Experimental; Tish. is Tishler.

TABLE V

Summary of intake of certain dietary factors in the case of the female experimental group of 135 patients

FACTOR	VERY UNSATISFACTORY		MINUS SATISFACTORY		(?) SATISFACTORY		SATISFACTORY-PLUS	
	Intake	N	Intake	N	Intake	N	Intake	N
Calcium.....	0.1- 0.39 g	58	0.4- 0.549 g	30	0.55- 0.69 g	22	0.7 g and up	25
Phosphorus.....	0.3- 0.89 g	77	0.9- 1.09 g	34	1.10- 1.30 g	19	1.3 g	5
Iron.....	4.0- 9.0 mg	94	10.0-11.0 mg	27	12.0 -14.9 mg	11	15.0 mg	3
Protein.....	25.0-44.0 g	24	45.0-54.0 g	39	55.0 -64.0 g	46	65.0 g	26
Animal protein....	0.0-26.0 g	38	27.0-35.0 g	56	36.0 -44.0 g	31	45.0 g	10
Vitamin A.....	500-1499 U*	8	1500-2999 U	77	3000-3999 U	34	4000 U	16
Vitamin C (anti-scorbutic).....			10-29 U	10	30-39 U	18	40 U	107
Vitamin C.....	0-19 U*	2	20-39 U	26	40-59 U	31	60 U	76
Milk.....	0-7 oz	16	8-15 oz	87	16-19 oz	11	20 oz	11

* Sherman units.

A part of the allowance of 1 gram of protein per kilogram must be protein of high efficiency to assure good maintenance of the body tissues. Partly for this reason, a number of authorities including Chaney and Ahlborn (1934) specify that the adult should have a pint of milk per day, and 2 different servings from a group of foods composed of

cheese, eggs, meat, or fish. This recommended daily ingestion would supply approximately two-thirds of the daily protein in the form of adequate protein or animal protein. On this basis the average woman of 56 kilograms would obtain at least 36 grams of animal protein, since it is often considered that an average serving of meat contains 17 to 20 grams of protein, 1 pint of milk about 16 grams, and 1 egg 6 to 7 grams.

Intakes lower than the standards are found for iron and vitamin A also. Fourteen of the 135 patients received 12 milligrams or more of iron daily; only 3 of these ingesting as much as 15 milligrams. Fifty patients fulfilled the standard for vitamin A of 3000 units (Rose, 1937).

Vitamin C was the only item studied which was well supplied in the diet. One hundred twenty-five patients consumed foods supplying 30 units (scurvy preventive) or more, while over one-half (76) took 60 units or more.

The pint of milk standard (or its calcium equivalent in cheese) was attained by only 22 of the 135 patients. Examination of the bivariate distribution between calcium intake and intake of the other dietary factors showed that when calcium was low the other factors were low. As calcium intake increased, the diets became less deficient in the remaining factors. Since milk, the most common calcium source, supplies protein, phosphorus, and vitamin A, one might anticipate the above finding; but milk contains so little vitamin C that increased calcium could not influence this factor.

A comparison between the 2 estimations of the Tishler dietary data showed an agreement of 89 per cent for calcium estimation, 84 per cent for protein, 75 per cent for animal protein, and 68 per cent for iron. The estimations of vitamin A and C intakes could be compared only very superficially since Hunt (Tishler, 1929) gave no specific number of units as her standards.

DISCUSSION

The number of cases in each male and female group, of both the Tishler and experimental patients, was not large. Since the degree of periodontal involvement of the cases in the Tishler and the experimental groups might not be comparable, and since there might be some question as to sex differences, it was inadvisable to treat the

data together. Therefore each of the designated groups was studied as fully as possible within the limitations set by the number of patients it included. The low frequencies in 3 of the groups made the use of certain statistical procedures inadvisable for them. As a consequence, much of the discussion is based on the results obtained from the more detailed study of the female experimental group, the only one composed of 100 patients or more.

Study of the probabilities given in Table I indicates significant association between periodontal condition and the intake of calcium, phosphorus, protein, animal protein, and milk. If approximately 90 per cent of all cases of periodontal disease are due primarily to local factors as has been suggested by Black (1920) and Gottlieb (Kronfeld, 1933), one should not expect such association in the case of patients who had no gross systemic disturbances requiring medical care. Calcium can act only as a systemic factor, especially since its predominant source in the diet is milk whose physical character provides, during consumption, no local stimulation or exercise to the dental tissues. The significant negative correlation between calcium or milk intake and periodontal disease may have some bearing on Hilming's (1938) finding that the most severe cases of periodontal disease in diabetic patients occurred in those forbidden milk.

Since, in the present study, it was found that only a small amount of the calcium in the diet was obtained from foodstuffs other than milk, it may be well to note that milk consumption in the United States is only a little over 1 cup per person per day (Dairy Council Digest, 1938) while in England it is just under one-half pint per person per day (Wishart, 1938).

Of the 135 female patients (Table V), 85 reported a daily average of less than Rose's standard of 3000 Sherman food units of vitamin A, and only 1 reported taking more than 6000 units, the standard suggested by the Bureau of Home Economics. Sixty-five of the 96 Tishler (male and female) patients ingested less than 3000 Sherman units. Without further mathematical consideration of the data, the low intake of vitamin A by these patients might be interpreted as a factor in the production of their periodontal disease. But when the probability of the observed association being due to chance alone is figured for the experimental female group, it is found to be 0.31, thereby dis-

crediting this deficiency in vitamin A as an important factor. Although vitamin A is known to have a specific influence upon epithelial tissue, there is as yet no incontestible evidence of the direct importance of vitamin A or of the actual amount necessary to influence periodontal tissues. Evidence presented herein suggests that use of vitamin A supplements is unimportant in the successful treatment of periodontal disease.

Contrary to popular opinion that "everyone is taking vitamin concentrates," it was noted that only 6 of the 135 patients in the female experimental group took vitamin A or D supplements. Because of the recognized influence of vitamin D upon calcium metabolism, it is unfortunate that up to the present time, no method has been devised for quantitative evaluation of this factor in human adults who are exposed to varying amounts of sunlight.

Vitamin C is frequently credited with being the most important factor in periodontal tissue condition. In this study, vitamin C was found, in most of the cases, to be supplied to the extent of the 30 unit scurvy-preventive standard. Nevertheless, gingivitis was present, despite the fact that only 23 out of the total of 280 patients had an intake of less than 30 units. Seventy-six of the 135 experimental females took more than 60 units (600 I.U. or 30 mg. ascorbic acid) of vitamin C. The probability (P) 0.64 supplies evidence that in this group of patients, exercising free choice of diet, vitamin C intake was not a significant factor.

The recorded vitamin C consumption was not exceptional in amount in light of the fact that the Tishler records, made 10 years previously, showed a similar result. Gillette and Rice (Sherman, 1937) estimated that the intake of vitamin C in the average family of the economic level with which the Association for Improving the Condition of the Poor deals, improved materially during the 15 year interval from 1914–1929. Daniel and Munsell (1937) state that "diets which provided at least the 'minimum daily requirement' for calories, protein, calcium, phosphorus, and iron, usually provided about 150 Sherman units (computed using the vitamin C values of uncooked foods) of vitamin C also." In spite of the rise in vitamin C consumption, a decreased incidence of periodontitis has not followed. However, it is conceivable that certain "limited" diets might be so lacking in

vitamin C that addition of small therapeutic amounts would be advisable in the treatment of periodontoclasia appearing in such patients. Yet Freeman and Glass (1939) found no correlation between blood ascorbic acid level and incidence of bleeding gums and gingivitis.

The consistencies found between the Tishler and experimental data were striking because of the great differences between the 2 groups. One group was composed mainly of clinic patients while the other was mainly private practice cases; one survey was made in 1927–1928 of patients living in the eastern United States sea coast area, while the other was taken approximately 10 years later of patients in the northern middle west area. Both showed low calcium and low vitamin A, and high vitamin C intakes, with no great differences in any of the factors studied.

Since the daily average for each dietary factor was computed in many instances from widely divergent daily intakes, there may be some question as to possible results if intakes had been more regular in amount. Work done on balance experiments has shown that the human system needs a period of adjustment to new intake levels. Therefore one may question the proportion of the dietary factor actually utilized by the body when the quantitative intake is highly irregular.

SUMMARY

1. Dietary records of freely chosen diets submitted by patients who applied for prophylactic or periodontal treatment at the dental school, supplemented by Tishler's data, were studied. The cases were divided into 4 groups, on the basis of sex and source of data. Thus, there were 135 females and 49 males in the 2 experimental groups, and 65 females and 31 males in the 2 Tishler groups. Conclusions are based on separate statistical treatment of each group. 2. Study of X^2 and P indicated little or no association between periodontal condition and age, vitamin A, vitamin C, iron, or acid-base excess. 3. Study of X^2 and P indicated association between periodontal condition and calcium, phosphorus, protein, animal protein, and milk intake. 4. The association between calcium and periodontal condition for a group of 135 females computed by the method of mean square contingency

was found to have the value of 0.5. Examination of the observed frequency in each cell as compared to the theoretical frequency, showed that the association was negative; that is, the lower the calcium intake, the greater the tissue involvement. 5. The coefficient of contingency of 0.5 probably underestimated the true association. 6. The coefficient of contingency for milk intake and periodontal condition in the group of 135 females was 0.44, as contrasted to 0.51 for calcium, indicating that computation or estimation of milk intake is not quite as valuable for prediction as computation of calcium. Yet the correlation coefficient of 0.95 between calcium and milk intake shows that the calcium in the diet comes predominantly from milk. 7. Inclusion of factors other than calcium in the prediction of periodontal condition results in values no greater than for calcium alone. Therefore, of the factors considered, i.e., protein, animal protein, calcium, phosphorus, iron, age, vitamin A, vitamin C, and acid-base excess, calcium is the most useful in prediction. These results were obtained when foodstuffs provided the source of the calcium. 8. Consistent results were found between the experimental and Tishler groups. 9. This study suggests that more cases of periodontal disease in human adults may have a systemic factor, in this case diet, than has been commonly stated by research workers. Therefore it is not considered feasible to separate periodontal disease into local and systemic categories.

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HORMONAL THERAPY FOR SOME GINGIVAL CONDITIONS[1]

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Hormonotherapy is widely applied in medical practice today, and there is substantial evidence to indicate that its use may prove efficacious in certain oral disorders. It is the purpose of this paper to suggest a basis for hormonotherapy in dentistry. Only the sex hormones, male and female, are considered.

Oral hormonotherapy reacts systemically, and its local use cannot be dissociated from its general medical aspects. Therefore, something must be said of the source of the hormones, their physiology, function and systemic relation.

Female sex hormone (*Estrogen*). Estrogenic substances have been recovered from the ovaries, placenta, urine, blood, faeces, bile, colostrum, testes, adrenals, pituitary and, according to Fisher, Kron and Zuckerman (28), from the sexual skin, the fluid of the sexual skin, and the liver of monkeys. It is widely distributed in nature, being found in all vertebrates, in some invertebrates, and in seeds, potatoes, female willow catkins; and in bituminous substances such as lignite and petroleum (1). It was recently shown that the addition of dilute solutions of crystalline estrone hastened the development of flowers in several plants, thereby demonstrating a possible relationship between animal and plant hormones (2). The primary action of the estrogenic hormone is to stimulate the growth and development of the female genital tract and mammary glands. It also stimulates the endometrium to growth and regeneration. It inhibits temporarily the growth of the primary and secondary genital organs in young males when given in large doses. It affects uterine motility and the pituitary gland (3, 4). The method of disposal of the estrogenic hormone is uncertain (5, 6).

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The names given to commercial preparations of the estrogenic hormone have led to confusion in its daily use. For this reason, the American Medical Association has recommended the following terminology (7):

1. Estradiol = dihydrytheelin = 3,17-dihydroxy $\Delta^{1,3,5,}$ estratriene.
2. Estrone = theelin = 3 hydroxy-17 Keto $\Delta^{1,3,5}$ estratriene.
3. Estriol = theelol = 3,16,17-trihydroxy $\Delta^{1,3,5}$ estratriene.

These preparations are on the market as amniotin, progynon, theelin, female sex hormone, oestrin, folliculin, follicular hormone, etc. Their effects are similar but not identical. Generally, no harmful results have been observed after either small or large dosages as ordinarily employed. Contraindications to the therapy include cases of abnormal uterine bleeding and cases in which there are or have been tumors of the primary or secondary sex organs (8).

The estrogenic hormone has a wide range of usage. It is employed chiefly to regulate disorders of the menstrual cycle and other gynecological conditions. It is also used in normal girls and women to induce breast development (9, 10, 11). It has been useful in treating children with vaginal gonococcal infection (12, 13, 14); in preventing postoperative adhesions and infections (15, 16); and as a nonspecific peritoneal stimulant to contribute to the safety of operations in the peritoneal cavity (17). Estrogenic hormone insufflation therapy has been reported an effective remedy in cases of atrophic rhinitis and ozena (18). The most noteworthy action of the estrogenic hormone on the oral cavity is to cause hyperkeratinization of the gingivae and oral mucous membranes, as well as keratinization of the mucous membranes in normally non-keratinized areas (19, 20). Other consequences are: the production of hyperplasia of the epithelium; and reduction or abolition of inflammatory infiltration in the corium. The oral epithelial hyperplasia and hyperkeratinization bear marked similarity to the results seen in atrophic rhinitis, and in the vagina.

Male sex hormone. The male sex hormone has been shown to be present in the testes, blood, epididymus, faeces, and urine of males, and also occurs in females and in plants. It is said to be synthesized in the body from cholesterol (21, 22, 23, 24). Its action has been studied by testicular transplants; by injections of testicular substances;

by injections and topical applications of extracts made from urine of human males and females, stallions, bulls, etc.; and by injections and topical applications of the synthetically prepared product. The extracted hormone is known as androsterone and was first prepared in crystalline form by Butenandt and Toherning in 1931. Butenandt then established its structural formula (25). Ruzicka, in 1934, was the first to synthesize the hormone from cholesterol, and in this form it is known as *testosterone*. The male sex hormone is available commercially under the names androsterone, virosterone, testosterone propionate, oreton and perandren.

The male sex hormone in castrated rats induces an increase in weight of the penis, Cowper's gland, preputial tissue and periurethral tissue (26). Testosterone causes growth of the mammary glands in normal and castrate male and female rats, inhibits growth of the thymus gland in immature animals, and hastens atrophy of the thymus in the normal adult. It overcomes loss of weight of the liver and heart following castration. In large doses it affects the pituitary gland and the female genital tract of castrate animals. Freud described an effect on the prostate (27).

Therapeutic experiences with the male sex hormone are limited, since commercial preparations were made available only recently. It is used in male impotence, infantilism, hypogonadism and benign prostatic hypertrophy, and in other sexual deficiencies. It has also been used in the treatment of alopecia areata and some forms of acne. Large doses are used for excessive and prolonged uterine bleeding in women. Harmful effects have not been reported.

The writer studied the effects of the male sex hormone on the gingivae and oral mucous membranes of patients of both sexes, and also of castrated and normal male and female rhesus monkeys. Grossly, the results were beneficial. Microscopically, the staining reactions were deeper than normal. The nuclei became more numerous, the nuclear membranes and the nucleoli more prominent, there being a greater number of cells with two or more. Mitosis was seen two or more times as frequently. The collagen appeared more solid and many more connective tissue nuclei were present. These findings will be presented in detail elsewhere.

In this resumé of the physiological actions and therapeutic indica-

tions of hormonotherapy, certain general factors must be emphasized. The effects are systemic whether treatment is topical or by injection, although injection produces a greater systemic reaction with like dosage. The effects are most marked during the period of therapy, although there is some degree of persistent effect (28, 29). Administering the secretion of a gland, while supplying the secretion and its effects, does not restore a defective gland to normal function. Where a specific result is desired, hormones may be given even though the source gland is functioning normally; e.g., to induce breast development in normal girls or to induce the growth of hair in otherwise normal males with alopecia areata.

PROCEDURE

In a previous report, the writer discussed the effects on the gingivae and oral mucous membranes of a group of women receiving hormonotherapy for gynecological disorders. The present study was concerned primarily with gingival involvements. Systemically, with the exceptions noted, the group was normal. All the patients had been referred for treatment after unsuccessful or incompletely successful treatment by other means over periods ranging from a few months to 2 or 3 years. Physical examination to detect contraindications preceded hormonotherapy, and during the course of treatment all the cases were kept under medical surveillance.

In addition to the physical examination, the routine for each patient included a preliminary biopsy, full mouth x-ray, impressions for study casts, and a detailed record of the gross gingival findings.

The hormones were injected into the buttock or the arm, or applied topically. The procedure for topical treatment was as follows: The patient was asked to bite into a cake of base-plate wax. A trough was formed by turning up and trimming the ends of the wax bite. This trough, filled with a salve prepared by incorporating the hormone in a lanolin base, was inserted in the mouth, held in position with the teeth closed, and allowed to remain in contact with the gingivae for 30 minutes. At the end of this time, most of the paste was absorbed. Some was lost by drooling, so that dosages could not be measured accurately.

The hormones used were Progynon B, Progynon-DH-Lanol (female follicular sex hormone), Oreton (Testosterone Propionate in a solution of sesame oil), and testolanol (male sex hormone). Four of the cases here reported are still under observation.

CASE REPORTS

The cases are summarized in Table I and are presented in detail below.

Case 1. M. B., a white female, married, aged 25 years. She complained of pus flow from the gingivae, and had received routine periodontal treatment for 3 months. Her menses began at age 14½, with regular periods lasting only 2 days; the flow was scanty, accompanied by severe cramps; pain in the back between periods. She has 1 child, has

TABLE I

Summary of results obtained with male and female sex hormone therapy in certain gingival disorders

CASE NO.	SEX	AGE	ORAL DIAGNOSIS	HORMONE USED	TOTAL DOSAGE	DURATION OF TREATMENT	ROUTE USED	RESULT	SIGNIFICANT MEDICAL FINDINGS
		yrs.							
1	F	25	Suppurative periodontoclasia	Progynon B	46,000 R.U.	6 weeks	Injection	No noticeable improvement	Scant menstrual flow. Two abortions. Overweight
2	F	23	Proliferative gingivitis	Progynon B	37,000 R.U.	16 weeks	Injection	Symptomatic improvement	None
3	F	36	Atypical lichen planus	Progynon B	47,000 R.U.	7 months	Injection	Symptomatic improvement	Dysmenorrhea following therapeutic abortion 4 years previous
4	F	22	Aphthous stomatitis	Progynon B	222,000 R.U.	16 months	Injection	Improved	Hyperthyroidism
5	F	44	Hypertrophic gingivitis with proliferation	Progynon B	150,000 R.U.	23 weeks	Injection	Gingivae restored to normal	Hysterectomy 10 years previous. Hypertension at present
6	M	16	Hypertrophic gingivitis and aphthous stomatitis	Oreton Testolanol Progynon	290 mg. 85 mg. —	3 months	Injection Topical Topical	Improved	Overweight
7	F	44	Desquamative gingivitis	Progynon-DH-Lanol	Approximately 150,000 R.U.	15 weeks	Topical	Excellent	None

had no miscarriages, but 2 abortions. She was 5 ft. 7½ in. tall and was overweight (170 lbs.). Her medical examination was otherwise negative.

Oral examination showed the gingivae generally of soft texture, unstippled, cyanotic, with uniformly deep crevices; there was some recession and blunting of the interdental papillae with little swelling; there were generalized deposits of calculus. Occlusion of the posterior teeth was normal; but the region of the anterior teeth showed an open bite. The maxillary and mandibular first incisors were widely separated. The maxillary left first incisor was extruded, mobile, and had a periodontal pocket 3 mm. deep with suppuration. There was slight suppuration around the other maxillary anterior teeth. The mandibular left No. 4 papilla[2] showed normal sections. The oral diagnosis was suppurative periodontoclasia. Estrogen (approximately 46,000 rat units over 6 weeks) was injected into the buttock with no noticeable improvement.

The dosage given in Case 1 was small. The writer has seen cases of this type improve while being treated with much larger doses for gynecological irregularities.

Case 2. G. G., a white female, single, aged 23 years. She complained of bleeding gingivae on toothbrush traumatization, and had received prophylactic treatment over a period of a year without effect. Her medical history and examination were negative.

Oral examination showed a full complement of teeth in good condition, with fair hygiene. The gingivae were generally normal in color, but the interdental papillae were swollen. Proliferation of the gingiva had caused recession of the normal gingival border so that a horizontal line of demarcation separated the normal gingivae from the pseudo papilla which was cyanotic, blunted, unstippled, shiny, hemorrhagic. All except a few anterior papillae were involved. *Microscopic findings:* Maxillary right No. 4 interdental papilla: keratin absent in the alveolar gingiva described as *pseudo papilla.* The surface of this area was irregular and ulcerated in appearance; the prickle cell layer was well-defined, the intercellular bridges were prominent. There was evidence of hyperplasia, mitosis was more frequent than normal, and the subepithelial tissue was densely infiltrated with chronic inflammatory exudate. The alveolar gingiva was otherwise normal. with the exception of a few scattered foci of inflammatory cells in the papillary layer, the areolar gingiva appeared normal. *Oral diagnosis:* proliferative gingivitis of undetermined origin. *Treatment:* Progynon B (37,000 rat units over 16 weeks) was injected with symptomatic improvement.

Case 2 was one of the first cases treated. The dosage was small. In the light of later experiences, it seems likely that larger quantities at each injection would have induced a more positive change.

Case 3. D. G., a white female, married, aged 36 years. She complained of soreness of the gingivae (especially of the lingual surfaces), hemorrhage on brushing, and soreness of the tongue. Her menses began at age 12, with fairly regular periods lasting 1 week; bleeding was profuse each day. Four years ago she had a therapeutic abortion after 4

[2] The method of identifying interdental papillae was as follows: between first incisors, central papilla; distal to first incisor, No. 1 papilla; distal to second incisor, No. 2 papilla; etc.

months of pregnancy. Periods have been irregular since abortion; duration 2 to 3 weeks; bleeding slight during first 2 weeks, becoming profuse in third. Periods were preceded by general malaise and severe supraorbital headaches, relieved by 3–6 aspirin or pyramidon tablets. For the past 3 years she has had constant swelling, redness, and a "drawing" sensation (without bleeding) at the gums around mandibular incisors. Symptoms worse during menstruation.

Oral examination showed the gingival tissues generally pale, not inflamed; papillae well formed, stippled; 1 pseudo papilla on maxillary left No. 3, and a slight marginal gingivitis in the mandibular molar region. Adherent violet-colored membranous patches, 2 to 3 cm. in diameter, were present in the premolar and molar regions and on the lingual aspect of maxillary anterior teeth. Patches resembled hypertrophic lichen planus and were confined chiefly to the alveolar gingivae, in places extending to gingival margin, except in the mandibular molar areas where they extended to the muco-buccal fold. *Microscopic findings:* Maxillary right No. 4 interdental papilla: well marked zone of keratin; epithelium markedly atrophic, with disappearance of epithelial pegs; prickle cell layer definite; intercellular bridges present but not well defined; dense chronic inflammatory exudate directly beneath epithelium. There was evidence of free hemorrhage in some places in the papillary layer, extending into the epithelium. Beneath the zone of chronic inflammation, connective tissue was fairly normal. Picture as a whole indicative of atypical lichen planus. Definite diagnosis of lichen planus cannot be made. *Oral diagnosis:* Undiagnosed gingival condition. *Treatment:* Estrogen (47,000 rat units over 7 months) was injected. Physical appearance of lesions was slightly improved with definite oral symptomatic relief during treatment. Menstrual periods were restored to normal, and subjective symptoms abolished. Eight months after the last injection the condition of the oral tissues was similar to that seen on original gross examination. The subjective symptoms had returned, but now appeared chiefly 1 week previous to the menstrual period. Periods were normal. Symptoms disappeared after menstruation.

This was also an early case, given small dosages over a comparatively long period of time. While oral symptoms were alleviated, it is doubtful if larger quantities would have cleared up this particular pathosis. The improvement noted here was primarily systemic—correction of the menstrual disorder. Mitigation of the subjective symptoms occurred concomitantly with the attainment of normality in the periods.

Case 4. A. R., a white female, aged 22 years. She complained of painful aphthous ulcers on mucous membranes lasting from 5 to over 30 days during the past 2 years. A new crop appeared before the old ones healed. She had been treated by dentist and physician without relief. Her menses began at age 13, with regular periods lasting 4 to 5 days; the flow was moderate, accompanied by cramps, backache, and headaches on first day. Her height was 64¼ inches, weight 113¾ pounds. Middle lobe of the thyroid was filled; skin delicate; distinct tremor of hands; breasts small, nipples inverted; pulse 126; blood pressure 110/70. Diagnosis: Hyperthyroidism.

Oral examination showed the gingivae of maxillary and mandibular anterior teeth

edematous, shiny, and purplish. Marginal inflammation was general; some pseudopapillae were present. *Microscopic findings:* Section of an aphthous ulcer: Epithelium bordering the ulcer was fairly normal. There was a dense inflammatory exudate consisting of polymorphonuclear and round cells in the corium of the ulcerated area. The mucous gland in the region of the ulcer was edematous, and infiltrated chiefly with plasma cells confined to the borders. The deeper layers of connective tissue appeared more nearly normal. *Oral diagnosis:* Aphthous stomatitis. The course of 35 aphthous ulcers was observed from January 1937 through May 1938. They persisted for from 5 to 38 days, appearing on lips, areolar gingivae, muco-buccal fold, occasionally on tongue, but never on alveolar gingivae. They appeared first as vesicular lesions which burst and left shallow, painful ulcers. Their onset was chiefly a few days to a week before or after menstrual periods; at times during middle of cycle. *Treatment:* Estrogen (222,000 rat units over 16 months) injected into the buttock; none in July, August, September, October, 1937. During the course of treatment, patient reported some decrease in the number of ulcers, less pain, more rapid healing. There was an entire absence of ulcers in July and August, with recurrence in September. The patient was ulcer-free for 15 days in December; for 3 weeks in February; for 10 days in March; for 3 weeks in April. The gingivae were improved.

At the outset the dosages for Case 4 were small; subsequently they were increased. Improvement was greater when the larger quantities were used. The desirable change produced may have been brought about by increased local tissue resistance rather than by striking at the causative factor. The underlying etiology is still obscure.

Case 5. B. M., a white female, married, aged 44 years. She complained of swollen, bleeding gingivae, and had received periodontal treatment by a general practitioner for several months, followed by several months of local treatment by a periodontist consisting of apoxesis, gingival curettage, instruction in home care and follow-up prophylaxis; some improvement noted. Her medical history showed a hysterectomy and bilateral salpingo-oophorectomy performed in 1927, with diagnosis of fibroid uterus. In March, 1937, at Presbyterian Hospital, her blood pressure varied between 180/120 and 220/120. There was some evidence of psychiatric tendency; fatigue was attributed in part to hypertension. Prognosis, favorable.

Oral examination in November, 1937, showed the free gingivae, buccal and lingual, and the interdental papillae to be swollen, and generally cyanotic over a depth of 2 to 4 mm. but most markedly in the maxillary anterior lingual area, extending onto the palate about 10 mm. There were several anterior shell crowns with overhanging margins. The interdental papillae were bordered by highly inflamed and deep red proliferative tissue which extended from the epithelium lining the gingival crevice. Gums bled easily but were not painful. *Microscopic findings:* Maxillary right No. 2 interdental papilla: Keratin layer in alveolar gingiva reduced to a thin line; absent in some areas. Granulosa cells not seen. Prickle cell layer was markedly hyperplastic; distorted near surface. Intercellular bridges near crest were not seen; otherwise definite. Epithelial pegs were long, pointed, frequently split. Some vacuolization. Similar epithelium in areolar gingiva except that pegs were not pointed. Collagen loose, densely infiltrated with chronic

inflammatory exudate composed almost entirely of plasma cells. Inflammatory reaction chiefly over alveolar gingiva, but occupying most of its extent, with a few scattered foci in the areolar gingiva. Blood vessels large and numerous; evidence of old hemorrhages in the papillary layer. *Oral diagnosis:* Hypertrophic gingivitis with proliferation, of undetermined etiology. *Treatment:* Estrogen (10,000 rat units per week for 5 weeks) was injected into the buttock with marked improvement in the gums after four weeks. Gingivae pale, but hard and firm without tendency to bleeding; no areas of proliferative tissue; slight marginal inflammation in anterior region. Eight weeks after injections were suspended, marginal inflammation and beginning proliferative tissue were again seen in mandibular left canine and maxillary left first and second incisor areas; interproximal papillae were normal. Parenteral treatment was resumed (10,000 rat units per week for 10 weeks). Improvement was noted after the second week. After the tenth week gingivae were apparently restored to normal. Patient reported a feeling of increased vitality during periods of treatment.

In Case 5 larger doses were used with a good result. Attention is called to the fact that intensive local treatment prior to hormonotherapy produced only partial improvement. With the temporary withdrawal of injections the proliferations returned, only to disappear promptly when estrogen was resumed.

Case 6. H. E., a white male, aged 16 years. He complained of swollen, easily bleeding gingivae, ulcerated at the margins, with recurrent crops of aphthous ulcers; gingival hypertrophy for the past three years. Has had local treatment consisting of prophylaxis, gum massage, and instructions in home care. His weight was 145¾ lbs. (overweight) and his height 65 inches.

Oral examination showed enlarged maxillary and mandibular anterior interdental papillae, most severe in region of incisor teeth. Papillae were cyanotic, rounded, thickened, shiny, and bled easily on palpation or trauma. There were surface ulcerations with sharply inflamed borders at the margins of some anterior teeth. *Microscopic findings:* Maxillary left No. 2 interdental papilla: The alveolar gingiva showed a thin keratin layer, ulcerated at crest; prickle cell layer normal; subepithelial tissue densely infiltrated with chronic inflammatory exudate occupying most of the extent of the alveolar gingiva. Epithelium of areolar gingiva normal; connective tissue edematous in papillary layer with scattered round cell infiltration. *Oral diagnosis:* Hypertrophic gingivitis of undetermined origin. *Treatment* (over 95 days): Oreton, parenterally, 290 mg.; testolanol, topically, estimated 85 mg.; progynon B (female sex hormone), 12 applications; gentian violet, 11 applications. There was symptomatic improvement, with the gingivae firmer, of better color, and not prone to bleeding. The interdental papillae were greatly reduced in size. Disappearance of marginal ulceration and aphthous ulcers was noted after the 68th day of treatment.

In Case 6 the therapeutic action of both male and female sex hormones was tested after local treatment had proved ineffectual. The best results were obtained with the male sex hormone in conjunction with gentian violet. The male sex hormone alone caused improve-

ment at the outset; there was a further improvement when the dye was introduced; and favorable progress continued after the gentian violet was stopped and the hormone was again employed alone. Only a slight response was evident with the estrogenic hormone.

Case 7. G. W., a white female, married, aged 44 years. She complained of sensitive, bleeding gingivae. She had received treatment in dental and dermatological clinics for over a year as follows: sodium perborate, zinc peroxide, baking soda, 2 per cent chromic acid, x-ray, liver extract injections, ABD capsules, apoxesis and prophylaxis. No improvement was noted. Vincent's smears were positive; mouth culture showed diphtheroids isolated anaerobically; streptococcus viridans predominated on the plate. Cultures and smears for monilia or fungus infection, blood smear, Wassermann, and repeat Wassermann were negative. Her menses began at age 13, with moderate flow and regular 28-day painless periods lasting 4 days. Medical examination showed no menopause symptoms.

Oral examination showed the maxillary and mandibular anterior gingivae red, raw, and denuded of surface epithelium to the mucobuccal fold; posterior and edentulous areas were also involved. *Oral diagnosis:* Desquamative gingivitis. *Treatment:* Progynon-dh-lanol (approximately 3,000 to 5,000 rat units per treatment) was applied topically 34 times in 12 weeks; following this period, less than 15,000 rat units were used in daily home application over 3 weeks. The last examination for the purpose of this paper, on July 14, 1938, showed the desquamated area on maxillary anterior labial gingivae reduced to 1 to 1½ mm. (its original extent was 8 mm. from margins to muco-buccal fold). There was a new mucous membrane, firm, adherent, continuous, and purplish in color. In mandibular anterior region the desquamation was only about ½ mm. in width. The edentulous areas were healed.

Case 7 is of especial interest because of the severity of the symptoms and the intensity and variety of the remedies attempted, without relief, before hormonotherapy; and because it is the only case in this group in which the topical method of application was used solely. Results in the beginning were spectacular. The healing started and progressed more rapidly on the areolar than on the alveolar gingivae.

Subsequent to a disturbance in the normal menstrual cycle (period 8 days late), which is a common systemic consequence of estrogenic therapy, treatment was suspended for 20 days. That is, several days before the regular period was anticipated, applications were stopped and not resumed until about a week after menstruation. Observation of the gingivae during this rest interval revealed first, that prior to the period the areas of desquamation increased in size, became more angry looking, and were accompanied by a burning pain; and secondly, that these signs remained until after menstruation when they promptly disappeared. Following the completion of treatment outlined in the

oral presentation of this report, the patient continued the home application of progynon-dh-lanol in small dosages for one year. At present the formerly desquamated areas are covered with a new mucous membrane. The gingivae appear for the most part normal. In some places the new tissue is thin, so that the redness shows through. Subjective symptoms have almost entirely disappeared. The gingivae become inflamed during the menstrual period, but subside into their improved state at termination of the period.

DISCUSSION

Promulgation of hormonotherapy may be based on the systemic and oral findings, which indicate that: **1.** Certain gingival irregularities occur during period commonly associated with hormonal change; e.g., the form of gingival hypertrophy often related to adolescence in both boys and girls (Case 6), the type seen chiefly in the middle aged (Case 7), and aggravations observed during the menstrual cycle (Cases 3 and 7). **2.** Both the estrogenic and male sex hormones act favorably on the gingivae, both grossly and microscopically. Substantiation is found in the report of Lewis, 1933, (12) of the success of estrogenic hormone therapy in clearing up chronic gonococcal vaginitis in children by promotion of maturation and keratinization of the vaginal mucous membranes, thus rendering them better able to resist infection. More recently (1937), Mortimer, Wright and Collip (18) described positive results in atrophic rhinitis and ozena by topical application of the estrogenic hormone. In their studies on the monkey, they found that the administration of the estrogenic substances produced a specific response in the conchal mucosa closely akin to that resulting in other "sex skin" areas. The work of Sprunt, McDearman, Raper, Rosahn, Hu, Pearce, Aycock, Kemp and Bainbridge (30, 31, 32, 33, 34) points to a distinct influence of the sex hormones on the permeability of tissues to both fluids and particulate matter. Following the administration in castrated male rabbits of the estrogenic hormone for 3 weeks, they injected india ink in one area and vaccinia virus in another. They concluded that ". . . the spread of India ink is much less than in the control animals. The resistance of the rabbit to vaccinia is increased."

The data of Ziskin, Blackberg and Slanetz (19) show that "bacte-

riological counts and smears disclosed a tendency of the mouth flora to become . . . more quiescent when estrin was injected . . . Treatment with estrin in ovariectomized monkeys . . . apparently made the gums resistant to mouth infections."

An analysis of the conditions under which the experiments reported in this paper were conducted, for the purpose of comparing them with the more flexible requisites of general practice, must take into consideration that whenever possible, supplemental aids to healing were not resorted to, in order that a more accurate evaluation might be made of the results of the hormone therapy alone. For example, when working with hormones, no other agents were used (see exceptions in case reports); local irritations (calculus, debris, overhanging margins, etc.) were permitted to remain; local therapy, in conjunction with hormonal, which might have accelerated progress, was avoided in all but one instance. Intangibles such as individual differences in patients, capacity of tissues to respond, cooperation of patient, all have a bearing on the final outcome.

Generally, it is our belief that where local therapy fails and an accessory is indicated, hormonotherapy may be instituted. In the light of our present knowledge, 2 ideas must be emphasized: (a) hormonotherapy cannot replace local methods; (b) the former should under no circumstances be undertaken without the cooperation of a physician both before and during treatment, even though we are aware of no deleterious systemic consequences. Finally, hormonotherapy generally is still in the experimental stage and any attempted use of these methods should be regarded in this light.

CONCLUSIONS

The male and female sex hormones exert a beneficial therapeutic influence on the gingivae and oral mucous membranes and may be employed successfully as an adjunct to local methods in some cases.

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DENTAL CARIES IN RATS PRODUCED BY HARD PILOT BISCUIT[1]

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In a previously reported study (Rosebury and Karshan, 5) of the dietary habits of 3 Eskimo settlements, no dietary characteristic or component appeared to be associated significantly with the prevalence of dental caries except pilot bread or ship's biscuit. This foodstuff was found to be used in greatest amount at the settlement (Eek) at which caries was most prevalent, in smaller amount at an orphanage at which less caries was found, and virtually not at all at the most primitive of the 3 settlements (Kepnuk) at which the incidence of caries was very low. It is generally agreed that dental caries is associated with the use of "white man's food." Pilot bread may be considered a prototype of white man's food. The analogous water biscuit, dating from the Stone Age, may represent man's earliest attempt to conserve the products of agriculture. Moreover, pilot biscuit resembles both physically and chemically the coarsely ground raw cereal previously found to be a primary causative agent of experimental dental caries in rats (2, 3, 8, 9). It seemed of interest therefore to determine whether pilot biscuit is capable of acting as a primary agent of rat caries under conditions similar to those previously employed with coarsely ground rice and corn.

METHODS

The experimental diets used are given in Table I. A control group of rats was placed on a corn-saccharose diet which is known to yield a relatively high level of dental caries (6). Of the 4 commercial varieties of biscuits used, "S" (soft), "H" (hard) and "M" (medium hard) were purchased locally, while "H2" (hard), obtained after the experiments with the other kinds had been started, is a variety used by white people and Eskimos

[1] Conducted with the aid of a grant for dental research from the Carnegie Corporation. A preliminary report was presented at the 15th General Meeting of the International Association for Dental Research, Baltimore, March 13, 1937 (*J. D. Res.* 16: 308, 1937).

in Alaska. Biscuits "H" and "H2" were of about equal hardness, and were the hardest of all the varieties examined. Biscuit "S" was distinctly the softest, and biscuit "M" was of intermediate hardness. Diets 112, 113, 114, 116 and 117 were of parallel composition, varying in the nature and particle size of the primary ingredient. In diet 117 rather finely ground biscuit was used as a particle-size control in the event that fissure caries appeared in the other groups, previous findings having shown that the ability of raw rice to produce fissure caries is overcome by fine grinding (4, 8). Diet 132 was started after the first results of the other groups had indicated that the incidence of caries produced by them would be low. In this diet very large particles of biscuit were given to the animals freely in a food cup while sugar alone was given separately in another, the large size of the biscuit particles making it impracticable to attempt mixing them in any fixed proportion.

TABLE I

Experimental diets

Diet No.	112	113	114	116	117	132
	Percentage					
Yellow cornmeal, 20–30 mesh*	82	—	—	—	—	—
Pilot biscuit "S" (soft) 10–20 mesh	—	82	—	—	—	—
Pilot biscuit "H" (hard)) 10–20 mesh	—	—	82	—	—	—
Pilot biscuit "H" (hard) through 60 mesh	—	—	—	—	82	—
Pilot biscuit "M" (medium hard) 10–20 mesh	—	—	—	82	—	—
Pilot biscuit "H2" (hard) through 3 mesh	—	—	—	—	—	ad lib
Saccharose	18	18	18	18	18	ad lib
Spinach leaves	5 grams per rat per day in all					

* "Mesh" refers to a screen having the indicated number of openings to the linear inch; "10–20" mesh means that the product was passed through a 10-mesh screen and retained on a 20-mesh screen, coarser and finer particles being discarded.

As in previous experiments, rats bred on a modified McCollum stock diet were distributed among the experimental groups at weaning, 22 days after birth. Groups of rats on each diet were killed at intervals of about 20 days after having been on the diets for about 35 days. The maximum experimental period was 100 days. In all groups of this series, including the coarse corn control (diet 112), decalcified celloidin sections were prepared from all the maxillary teeth and from one side of the mandible. In most instances, in addition, a ground section was prepared from the other side of the mandible. Both decalcified and ground sections were stained with hematoxylin and eosin. Identification and rating of lesions were made microscopically, in accordance with the scale previously described (3, 9), in which each carious fissure is scored from 1 to 10 depending on its size and degree of penetration. The "index" of caries (see Table II, footnote) is based on these values. Proximo-gingival caries lesions, described below, were rated according to the same scheme, except that since in this instance enamel was generally

involved only secondarily, the stage 3 lesion (showing moderate infiltration of the dentin surface) was the smallest that could be identified with certainty.

RESULTS

Lesions of the teeth observed in these animals were of 3 varieties, cusp-fracture lesions, fissure caries and proximo-gingival caries.

Cusp-fracture lesions occurred only in the coarse corn control group. These lesions can usually be seen without difficulty with the unaided eye or under low magnification in the gross specimen. They correspond with most of the "macroscopic caries" of other observers, as noted elsewhere (9, 10). The findings in previous studies (6, 7, 8) indicate that their etiology is distinct from that of the other types of lesions of rat teeth. Since they develop by mechanical fracture of the exposed dentin at the cusp surface under the impact of hard food particles and become "carious"—that is, infected—only secondarily, they are not considered analogous to dental caries in man. The biscuit particles used in these experiments were evidently not hard enough to fracture the teeth, since no lesions of this type were found in the biscuit groups.

Fissure caries occurred in both groups, much more commonly among the animals that received corn than among those that received biscuits. These lesions, which seem to be entirely comparable with caries of the pits and fissures of human teeth, have been described in detail and illustrated in previous reports (3, 8). Fissure caries produced with biscuits, shown in *figs. 1, 2 and 3*, is identical with that produced with coarsely ground rice or corn.

Proximo-gingival caries also occurred in both corn and biscuit groups; but in contrast to fissure caries it appeared more commonly among the animals given the biscuit diets. In previous studies with rice and corn diets caries of this type has rarely been seen. The data for diet 112 (Table III) show that with coarse corn as the caries-producing agent, proximo-gingival caries accounted for only about 4 per cent of all the lesions, and that these were limited to the maxillary teeth. In previous studies only the lower teeth were examined routinely. Caries of this type occurred on approximating surfaces of teeth in all but 1 instance (diet 117), in which a lesion developed on the exposed mesial surface of a mandibular first molar.

Examples of proximo-gingival lesions produced with the biscuit

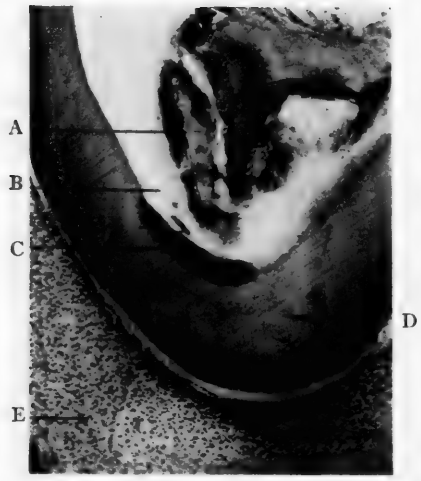

FIG. 1

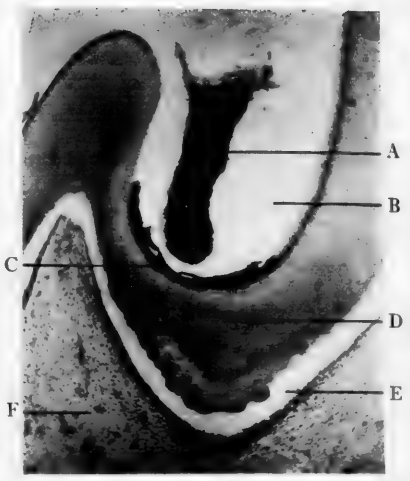

FIG. 2

FIG. 1. Fissure caries produced with pilot biscuit. A, food mass impacted in fissure; B, enamel (lost in decalcification); C, characteristic spread of caries at junction of dentin and enamel; D, dentin; E, pulp. Rated as stage 3. Rat 1615, Diet 114.

FIG. 2. Fissure caries produced with pilot biscuit. A, food plug impacted in fissure; B, enamel (lost in decalcification); C, spread of caries at dentino-enamel junction; D, dentin; E, dentinoid, showing imperfect calcification; F, pulp. Rated as stage 3. Rat 1880, Diet 132.

FIG. 3. Fissure caries produced with pilot biscuit. A, carious infiltration of dentinal tubules; B, food plug in fissure, broken in sectioning; C, residual enamel matrix, suggesting diffuse caries of enamel; D, dentin; E, uncalcified dentinoid; F, pulp. Rated as stage 5. Rat 1884, Diet 132.

diets are given in *figs. 4 and 5*. The lesion usually starts at the junction of enamel and cementum just below the gingival termination of the contact area. The gingival papilla is depressed by impaction, in the interdental space, of a mass of debris, probably consisting of food and bacteria. Enamel is usually not primarily involved, the

FIG. 4

FIG. 5

FIG. 4. Proximo-gingival caries produced with pilot biscuit, affecting both of an approximating pair of teeth. A, pulp; B, dentin; C, enamel (lost in decalcification), with surface debris displaced in sectioning; analogous structures on the other tooth have been lost; D, carious infiltration of dentin; E, caries spreading along the dentino-enamel junction; F, food debris; G, gingival papilla, normally filling the interdental area under the enamel contact, here depressed, presumably by the impacted food mass. The lesion shown at the left is rated as stage 6, that on right as stage 5. Rat 1883, Diet 132.

FIG. 5. Proximo-gingival caries produced with pilot biscuit, affecting both of an approximating pair of teeth. A, depressed gingival papilla; B, pulp; C, dentin; D, impacted food mass; E, carious dentin; F, enamel, lost in decalcification. The lesion shown at the left is rated as stage 8; that on right as stage 7. Rat 1833, Diet 117.

earliest apparent change being infiltration of the cementum and dentin; but the enamel may be undermined as the infectious and disintegrative process spreads along the dentino-enamel junction. The changes characteristic of caries appear in the dentin.

Table II presents a general summary of the incidence of carious lesions among the different experimental groups. The coarse corn

control group (diet 112) had a characteristic caries index of 7.7, with lesions in 13 of the 14 animals and in 57.8 per cent of the teeth examined. The maxillary and mandibular teeth were affected in about equal proportions. Caries in this group was almost exclusively of

TABLE II

Dental caries in rats fed various biscuit diets and a coarsely ground corn diet

DIET GROUP	NUMBER OF ANIMALS	NUMBER OF TEETH EXAMINED	EXPERIMENTAL PERIOD	CARIES INCIDENCE			MAXILLARY MOLARS		MANDIBULAR MOLARS	
				Percentage of		Index*	Fissure caries	Proximo-gingival caries	Fissure caries	Proximo-gingival caries
				Animals	Teeth		Percentage of Teeth			
			days							
112	14	166**	29–100	92.9	57.8	7.7	58.3	6.0	56.7	0
132	11	99***	78–100	72.7	20.2	2.8	9.1	12.1	18.2	0
114	14	168	35–100	57.2	10.7	1.5	2.4	8.3	6.0	4.8
117	13	153**	32–100	46.2	19.6	3.7	0	19.2	1.3	18.7
116	11	129**	26–100	36.4	3.1	0.3	0	1.5	4.7	0
113	13	156	35–100	0	0	0	0	0	0	0

* The index in these groups was calculated by dividing the total for the group of the scores as rated for each lesion by one-third the number of teeth examined, making the indexes equivalent to those previously reported, in which only 3 teeth per animal were examined, and the divisor was the number of animals in the group.

** Several mandibular third molars were lost in grinding.

*** Ground sections of mandibles were not prepared in this group.

TABLE III

Comparative frequency of fissure caries and proximo-gingival caries in rats on biscuit diets and a coarsely ground corn diet

DIET GROUP	NUMBER OF TEETH EXAMINED	FISSURE CARIES LESIONS			PROXIMO-GINGIVAL CARIES LESIONS			ALL CARIES LESIONS		
		Number	Percent*	Index**	Number	Percent*	Index**	Number	Percent*	Index**
112	166	108	65.0	7.3	5	3.0	0.4	113	68.0	7.7
132	99	13	13.2	1.9	8	8.1	0.9	21	21.2	2.8
114	168	7	4.2	0.6	12	7.1	0.8	19	11.3	1.5
116	129	3	2.3	0.2	1	0.8	0.1	4	3.1	0.3
117	153	1	0.7	0.1	34	22.2	3.6	35	22.9	3.7
113	156	0	0	0	0	0	0	0	0	0

* Ratio of number of lesions to number of teeth examined, expressed as percentage.

** See first footnote under Table II.

the fissure type (excluding fracture lesions); and the few proximo-gingival lesions were limited to the maxillary teeth.

Caries appeared in 4 of the other 5 groups, which received biscuit diets. Its incidence on the whole was low compared with that in the coarse corn group. The comparative incidence of fissure caries and proximo-gingival caries is given in Table II in terms of percentage of teeth affected, and in Table III in terms of the number of individual lesions in each group, the ratio of this number, expressed as percentage, to the number of teeth examined, and the index. Only the index

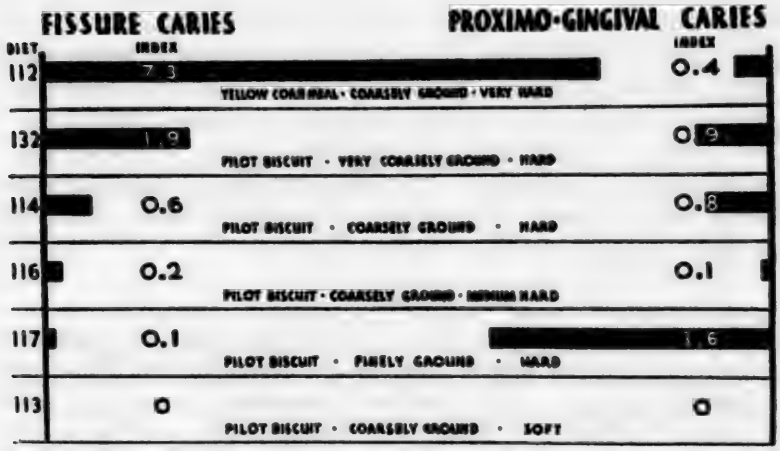

FIG. 6. Distribution of fissure caries and proximo-gingival caries in rats on coarse corn and various biscuit diets. Index values, shown graphically, are measure of both number and relative size of lesions; but similar distribution is shown in percentage values (Table III), which are measure of number of lesions only.

includes a measure of the relative extent as well as the number of the lesions. The relation of the index values for the 2 types of lesions in the different groups is shown graphically in *fig. 6*.

From these data it will be seen that the highest incidence of fissure caries among the rats on biscuit diets occurred in the group that received the largest particles of one of the hard biscuits (diet 132). The group that received the other hard biscuit ground to smaller particles (diet 114) had less fissure caries; and the group that received the biscuit of medium hardness, ground as in the last instance (diet

116), had still less fissure caries. The soft biscuit, ground as in the 2 preceding instances (diet 113), yielded no caries. Thus both increasing hardness of biscuit and increasing particle size appear to have produced increasing levels of fissure caries. These findings are in agreement with those previously reported for rice and corn diets (4, 6, 8).

The incidence of proximo-gingival caries, on the other hand, showed a somewhat different pattern. Its highest incidence occurred in the group (diet 117) that received one of the hard biscuits ground more finely than was done in any of the other groups. This diet produced less fissure caries than any of the other caries-producing diets, a finding in line with those given above. The other 2 biscuit diets (diets 114 and 132) containing more coarsely ground hard particles, produced distinctly less proximo-gingival caries than diet 117. The diet containing biscuit of medium hardness (diet 116) produced less proximo-gingival caries (as well as less fissure caries) than those with harder biscuits; while that containing the soft biscuit (diet 113) produced no caries of either variety.

DISCUSSION

Proximo-gingival caries in rats, which occurs rarely on coarse rice or corn diets but, as these results indicate, in comparative abundance on hard biscuit diets, may or may not be analogous to proximal caries in man. The rat lesion differs from human proximal caries in that the former attacks cementum primarily, the latter enamel. This difference may depend on the differing anatomy of the interdental area of the rat as compared with man. Proximal caries, like fissure caries, affects young human beings predominantly. In the young human denture the area of interdental contact is very small and is situated at about one-third the distance from occlusal plane to cemento-enamel junction. The proximal surfaces of the crowns slope from the occlusal plane to the contact to form a V-shaped cleft resembling the fissures of the occlusal surface. In the rat, on the other hand, the area of interdental contact is large, extending from the occlusal plane or a point near it to include about two-thirds of the height of the crown. Little or no cleft is present at the occlusal plane, the relationships being distinctly different from those of the fissures.

In both man and rat, however, the proximal tooth surfaces diverge on the root side of the contact, forming a cleft which is normally completely filled by the gingival papilla enclosing the interdental septum of bone. In the rat (*figs. 4 and 5*) proximo-gingival caries evidently results from impaction of a food mass into this gingival area, depressing the gingiva, inducing resorption of underlying bone, and consequently making space for the retention of the food mass, where it can undergo fermentation in contact with the tooth surface. In the rat the cementum of the root just below the cervix is affected first, perhaps by anatomical accident. In man the development of proximal caries probably follows a similar course, but because of the greater extent of enamel surface on the root side of the contact, the impaction area itself being relatively much smaller in scale, only enamel is exposed to the acid products of the fermentation. It may be noted that the interdental relationships in the rat resemble those found in older human beings in whom the occlusal surfaces of the teeth have become flattened by marked attrition. In such persons a type of proximal caries occurs that seems identical with proximo-gingival caries in rats.

The data given above indicate that the primary etiology of rat proximo-gingival caries differs somewhat from that of fissure caries. They suggest that hardness of biscuit influences the development of both varieties of caries, but that particle size either is not a factor in proximo-gingival caries, or else that its effect is the reverse of that observed with fissure caries, since the diet containing the smallest particles (diet 117) produced the highest incidence of proximo-gingival caries. It may be noted, on the other hand, that proximo-gingival caries did not develop in previous experiments (4, 6, 8) in which cooked or finely ground cereal was used in place of the raw and coarsely ground product. While the precise conditions that determine the production of these lesions cannot yet be stated, it seems clear that like fissure caries they require certain particular physical characteristics in the causative food, and that, indeed, their primary etiology is related to that of fissure caries (although not identical with it), since in the present experiments both types of lesion were produced with the same food.

The production of experimental caries with pilot biscuit, including

among the more effective types of biscuit one that is used by Alaskan Eskimos, lends weight to the evidence previously adduced (5) which incriminates pilot biscuit as a cause of dental caries among Eskimos. If the essential similarity of proximal caries in man and proximogingival caries in rats be assumed, it seems the more significant that among the Eskimo subjects of the previously reported study (11) occlusal (fissure) and proximal caries preponderated more than ordinarily, occurring almost to the exclusion of other types.

On the whole the parallel between dental fissure caries in man and its experimental counterpart now seems fully satisfactory. It was shown previously that the 2 are virtually identical in their histopathological characteristics (3, 8) and also that they agree closely in such aspects of their etiology (4, 6, 7, 9) as the mitigating influence of vitamin D, which in the rat are clearly secondary or modifying agencies, affecting only the amount or degree of severity of the disease, which is produced primarily by another causative condition. The data for rats define this primary causative condition unequivocally. Caries in rats results directly from the mastication of certain individual foodstuffs exemplified by coarsely ground raw cereal particles or hard pilot biscuit. Heretofore, since human beings were not known to use food exactly comparable to the coarse cereals effective in rats, this principle of primary etiology could not with confidence be applied to man. This difficulty has evidently been overcome by the finding that pilot biscuit is capable of acting as a primary agent of dental caries in rats. For pilot biscuit, physically and chemically similar to the raw cereal particles, is in itself a foodstuff used by man, and may moreover be considered representative of a widely used class of human foods with similar composition and physical characteristics.

The determining characteristics of these foods seem to be (a) hardness, necessitating strong masticating pressure; (b) compactness, preventing their rapid softening in the presence of moisture, both properties inducing forcible and therefore durable impaction of the particle into crevices of the teeth; and (c) a high content of carbohydrate, supplying substrate for bacterial fermentation with acid products. Other characteristics shared by raw cereals and biscuits may possibly also be significant, such as their low content of protein and basic elements.

The clinical data on Eskimos previously reported (11), augmented by the bacteriological and chemical findings on their saliva (1), demonstrated that caries among Eskimos is the same disease as caries elsewhere. This fact, coupled with those given above, points to the inference that foods with the characteristics of pilot biscuit may be primary causative agents not only of caries among Eskimos, but of dental caries in general. The data as a whole suggest that the principle of primary causation of caries derived from studies of the rat may be directly applicable to man.

SUMMARY

Seventy-six rats, in 6 groups of 11 to 14 animals, were placed at weaning on (a) a diet of coarsely ground yellow corn, saccharose and spinach, and (b) 5 diets in which the corn was replaced by pilot biscuit of four varieties differing in hardness, the diets also differing in particle size of biscuit. The animals were given the diets for periods ranging up to 100 days. Nine or more of the 12 molars of each animal were examined for caries by means of histological sections.

The group that received coarsely ground corn showed a characteristically high incidence of dental caries, accompanied as in previous experiments by cusp-fracture lesions. Caries lesions were about equally distributed among maxillary and mandibular molars.

Among the rats on biscuit diets, caries occurred in 4 of the 5 groups, but cusp-fracture lesions were entirely absent. The lesions included both fissure caries and proximo-gingival caries, which is described and illustrated. Both types of caries were produced in greatest amount by the harder biscuits, in lesser amount by the biscuit of medium hardness, and not at all by the softer biscuit. Fissure caries was produced in greater amount by coarsely ground than by finely ground hard biscuit, confirming previous findings with raw cereal. On the other hand the highest incidence of proximo-gingival caries occurred on the diet containing hard biscuit finely ground (through 60-mesh), indicating that their primary etiology differs in detail from that of fissure caries.

The relationship of proximo-gingival caries in rats to proximal caries in man is discussed. The findings add important evidence that fissure caries in rats is the true counterpart of fissure caries in man, and

in conjunction with other data point to the principle that individual foods, of which pilot biscuit is a type, may be primary causative agents of dental caries in man.

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SOME CONDITIONS THAT EFFECT THE ODOR CONCENTRATION OF BREATH[1]

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Fetor ex ore, or unpleasant breath has long been a common condition in man. Although the condition is known to be quite prevalent and is thought to be a reflection of some systemic condition very little data concerning the source and cause is available. Although it has been considered a valuable aid in diagnosis (1) (2), before it can become an accurate diagnostic aid more evidence concerning its source and cause should be found.

From a theoretical point of view breath can be contaminated from three primary sources. The odors can be derived from the mouth, the lungs, and the nasal passages. Putrefaction processes in the mouth such as stagnant saliva, decaying teeth, pyorrhea, and degenerated cells on the tongue may give rise to odiferous substances which would contaminate the breath. Digestive disturbances in the stomach and small intestines may give rise to volatile substances which after absorption into the blood stream would be absorbed in alveolar air and excreted in the breath. Any putrefaction process in the lungs or in the nasal passages would also give off odiferous substances during the excursion of air in the process of breathing.

In a recent paper (3) the authors described a method whereby the odor intensity of breath, lung air, and the odors derived from the mouth could be determined. By specialized apparatus the odors from the various sources were segregated, frozen in liquid nitrogen to concentrate them, and the intensity determined by means of an osmoscope (4). By these methods the odor intensity was given a significant mathematical term that could be used for statistical pur-

[1] This work was conducted under a grant from the Colgate-Palmolive-Peet Company. Presented at the 17th General Meeting of the International Association for Dental Research, Cleveland, Ohio, March 18–19, 1939 (*J. D. Res.*, 18: 255, 1939).

[2] Colgate Research Fellows.

poses. By the use of these methods total breath odors and mouth odors were determined and the effect of various factors were evaluated.

EXPERIMENTAL

Patients were selected with no putrefactive lung or nose disorders. This was done to eliminate these conditions in the general breath samples as they would interfere with the correlation of mouth condi-

TABLE I

Average odor intensity

	BREATH pO			MOUTH pO		
	Agg.	M.	F.	Agg.	M.	F.
No. Cases	95	62	33	200	128	72
Av. pO	2.0	2.0	2.0	1.92	2.11	1.57

TABLE II

Average pO value for age groups

	20 YEARS AND UNDER			21–50 YEARS			51 YEARS AND OVER		
	Aggr.	M.	F.	Aggr.	M.	F.	Aggr.	M.	F.
Breath pO									
No. cases	12	4	8	75	51	24	11	7	4
Av. pO	1.30	1.22	1.33	1.98	1.94	2.08	2.72	2.85	2.50
Mouth pO									
No. cases	20	8	12	144	95	49	36	25	11
Av. pO	1.40	1.68	1.20	1.85	2.01	1.55	2.45	2.62	2.09

tions and breath odors. The age, sex, time after meal, gingivitis, pyorrhea, and caries were recorded. The amount of gingivitis, caries, and pyorrhea were determined by clinical examination, and were recorded in terms of $-$, $+$, $++$, $+++$, by means of an empirical scale. The same people made the examinations throughout the work. For this reason, although the estimations were qualitative they may be considered quite accurate. General breath samples on 98 patients and mouth odors of 200 patients were determined and the above con-

ditions were correlated with the odor concentration found. The results are in Tables I to VI.

Average odor intensity. It has been found that a pO value under 2 is not offensive to most people while 2 or above would be considered objectionable. On this basis the average breath odor of the cases compiled is just on the border line between offensive and non-offensive. It was found that 56 per cent of the cases tried had a pO value of 2 or above. This would indicate that slightly more than half of the people have offensive breath at any time during the day. The average odor intensity arising in the mouth is slightly under that which is considered offensive; 47 per cent of these cases have a pO value of 2 or more and can be considered as offensive.

The effect of age: It can be seen from Table II that age is a decisive factor in both general breath odors and mouth odors. It would seem

TABLE III

Average pO value per time after meal

	BREATH pO					MOUTH pO			
TIME	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	2 hrs.	3 hrs.	4 hrs.	5 hrs.
No. cases	8	14	30	19	8	31	76	42	17
Av. pO	1.12	2.50	2.16	1.89	2.37	1.50	1.98	1.82	2.64

that in both cases the odor intensity increases with age and that bad breath is quite prevalent in older people. Although there is an insufficient number of cases in the high and low groups of the breath odor samples to draw any definite conclusions, the trend is as one would expect. There is usually a retrogression of the body processes with age which may cause an increase in breath and mouth odors. With a recession of the gingival tissue there is more area in which food may impact with the subsequent putrefaction and production of odors. Furthermore, there is a greater tendency toward pyorrhea in these cases which would in itself cause an increase in odors.

The effect of time after meals: It is a common opinion that hunger may be associated with breath and mouth odors. This may be explained on the basis that stagnation may occur in the mouth and as the mouth has not been cleansed by the excursion of food the odor

intensity should be higher. As can be seen from the table the odor intensity of both general and mouth samples increases with time after meal.

The effect of caries: The data presented on the effect of caries shows conclusively that the incidence of dental caries has a marked effect on mouth and breath odors. The average odor intensity in which no

TABLE IV

Relation of pO value to caries

	BREATH pO				MOUTH pO			
CARIES VALUE	–	+	++	+++	–	+	++	+++
No. cases	42	28	12	13	37	82	54	21
Av. pO	1.65	1.86	2.63	3.08	1.54	1.99	2.12	2.0

TABLE V

Relation of pO value to gingivitis

	BREATH pO				MOUTH pO			
DEGREE OF GINGIVITIS	–	+	++	+++	–	+	++	+++
No. cases	56	22	9	8	80	69	29	10
Av. pO	1.63	2.43	2.25	3.14	1.33	2.23	2.55	2.20

TABLE VI

Relation of pO value to pyorrhea

	BREATH pO				MOUTH pO			
DEGREE OF PYORRHEA	–	+	++	+++	–	+	++	+++
No. cases	68	15	5	6	103	24	27	28
Av. pO	1.68	2.66	2.80	2.83	1.48	2.08	2.62	2.84

caries were present showed a very low pO value which rapidly increased with the incidence of caries. This is as one would expect, as carious lesions furnish many places for the retention and putrefaction of food particles.

The effect of gingivitis: Gingivitis may give rise to putrefactive odors as infection is present in these cases. The results indicate that this is true. There is a marked increase in both general and mouth odor intensities.

The effect of pyorrhea: As would be expected, pyorrhea gives rise to a foul odor that is reflected in both general and mouth odor intensities.

The effect of sex: The general breath samples do not show a difference in odor intensity between the sexes when the total of the two groups are averaged. There is, however, a difference in the pO values of the various age groups. The increase in odor intensity with age seems to progress more rapidly in women than in men. This may be due to the fact that women mature more rapidly than men. This correlation does not hold in regard to the mouth odor samples. In both the average pO values and the increase in odor intensity with age we find that the mouth odors are appreciably less in women. On the basis of these results it would seem that women are more concerned about mouth hygiene than men.

DISCUSSION

Dentists have recognized for some time that breath odors may in many cases arise in the mouth and that many mouth conditions influence these odors. On the other hand, there are numerous cases in which mouth conditions play very little part in the production of breath odors. In so far as the dentist is concerned odors arising from sources other than the mouth cannot be influenced to any appreciable extent by oral hygiene and prophylaxis. For this reason the effect of the above variables are of prime importance. It would seem that any pathologic condition of the mouth is definitely a variable in the problem of mouth and breath odors and that oral hygiene and operative procedures in the mouth may play a definite and constructive part in the alleviation of bad breath, particularly in regard to those odors arising in the mouth. On the basis of the above statistics the mouth conditions that produce breath odors are in the order of their importance, pyorrhea, gingivitis, and caries.

CONCLUSIONS

Pyorrhea, time after meals, age, sex, caries, and gingivitis are variables in the production of breath and mouth odors.

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THE CHEMOTACTIC INFLUENCE OF HUMAN SALIVA UPON LEUKOCYTES[1, 2]

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This paper is designed to open studies upon the chemotactic influences, possessed by saliva, for leukocytes. When living organisms capable of motility are influenced by a chemical stimulus so as to cause their movement to be directed toward or away from the source of the stimulus the phenomenon is called chemotaxis. This phenomenon is conditioned by the necessity for unilateral stimulation; the organism must be within a concentration gradient of the substance acting as stimulus if it is to be influenced. The discovery of chemotaxis is attributed to Engelmann (1868) while Leber (1888) was the first to study the reaction of leukocytes to chemical stimuli. The only observations to date on the chemotactic property of saliva have been made by Hugenschmidt. He found that when capillary tubes containing saliva were introduced into the peritoneal cavity of a guinea pig, leukocytes entered the tube even forming dense plugs 2 mm. long. It was also observed that the attraction exerted on the leukocytes was directly related to the bacterial population of the saliva. He concluded that this fact had a possible bearing on the relative freedom of the oral cavity from traumatic infection. The presence of leukocytes in the human oral cavity has been noted under various conditions, normal and abnormal. Miller (1904) repeatedly observed phagocytosis in extraction wounds and thought it probable that this phenomenon materially contributed to the fact that these wounds usually are clean and free from infections. Mendel (1917) made differential counts of leukocytes from the gingival crevice and

[1] Presented at 75th Annual Midwinter Meeting of the Chicago Dental Society, February, 1939.

[2] Part of the expense incident to this study has been met by an appropriation from the Pennsylvania College of Dental Surgeons Fellowship Fund.

observed their phagocytic behavior. He reported differences between normal and "pyorrheic" individuals, and believed he could recognize a "prepyorrheic" state. Ruggieri (1928) studied the source and survival of leukocytes in the mouth. He believed they reached the surface by diapedesis from the capillaries of the gingival submucosa, especially following the use of the tooth brush. They show ameboid motion and are phagocytic for relatively long periods of time. Comroe (1934) found that counts of salivary leukocytes varied tremendously both in different individuals and in the same individual at different times. In artificially produced leukocytosis (adrenalin) he usually found an increase in salivary leukocytes. The significance of these findings in the physiology of the mouth can as yet only be surmised. Weinmann (1936) reported that equal amounts of leukocytes produce more proteolytic enzyme in mouths of persons immune to caries than in the mouths of caries susceptibles.

(a) The claim by Hugenschmidt that saliva attracts leukocytes, (b) the presence of these cells in the saliva and on the oral mucosa under various conditions, and (c) the importance these cells frequently assume in the pathologic processes are 3 facts calling for a critical reconsideration (by newer methods) of the chemotactic properties of human saliva. The present paper reports the first phase of such a reconsideration.

MATERIALS AND METHODS

The cells used in these tests were motil leukocytes obtained by injecting a rabbit intraperitoneally with *ca.* 300 cc. of 0.89 per cent NaCl; 4 hours later it was possible to withdraw a leukocyte-rich exudate from the peritoneal cavity. When tubes containing the leukocyte suspension were packed in ice, the cells were potentially motil for 5 hours. It was desirable to have the exudate free of bacteria and red blood cells.

Observations have been made on the salivas of 3 adult individuals (A, D and S). Subjects D and S were females of *ca.* the same age i.e. 27 years, while subject A was a male of *ca.* 50 years. A chronic gingivitis was present in A's mouth but D and S gave a normal oral picture.

Unstimulated "mixed" saliva of the oral cavity was used in these experiments. Five minutes previous to the collection of the saliva specimen the mouth was thoroughly rinsed for 1 minute with *ca.* 3 cc. of steril distilled water. The samples were collected in steril test tubes equipped with steril funnels and covers. Since it has been shown that leukocytes react to even very slight stimuli special attention has been paid to the cleaning and handling of materials used in test. When about 5 cc. of saliva were obtained the funnel was removed and the test tubes rotated vigorously to insure thorough mixing. Cells, debris and some of the bacteria were removed from the saliva by light centrifugation for

10 minutes. A drop of the supernatant fluid was transferred to a clean slide by means of a steril pipet. The tip of a microcapillary tube measuring *ca.* 50 micra in diameter was introduced into the drop of saliva. When the liquid had filled the tube, the free end was sealed with petrolatum. The tip of the microcapillary tube which had been in contact with the saliva was then broken under a drop of exudate; this precaution was taken to prevent the obstruction of the pipet by air bubbles. The tube was transferred to a fresh drop of leukocyte-containing exudate. A cover slip was lowered onto the drop and the preparation sealed with petrolatum to prevent drying. The slide was placed on the stage of a microscope in a warm box at 37°C. Temperature was maintained to ±1° with a thermostatic control. The preparation was allowed to warm for 15 minutes in order that the cells might resume their ameboid movements. The paths traced by the leukocytes were plotted on paper by camera lucida. Directional movements of the leukocytes were studied over a 10 minute period. Control tests were set up using pipets filled with substantially cell-free, supernatant fluid obtained by centrifuging the exudate.

Results indicate that a 10 minute period of observation constitutes a satisfactory sampling. Since it is not feasible when observing the movements of a number of leukocytes simultaneously to plot continuous paths, the position of each leukocyte was recorded at 1 minute intervals. The points representing the successive position of each cell were connected by straight lines.

RESULTS AND DISCUSSION

Fig. 1 represents a camera lucida drawing of a microscopic field containing several leukocytes and at the left the tip of a micropipet. The saliva of 1 of the subjects studied is used as the test substance in the pipet. The initial position of the leukocyte is represented by a ring, and a line designates the path traversed by the leukocyte during observation. Of 8 cells observed, 7 showed movement in the direction of the pipet. For the purpose of comparison *fig. 2* is introduced as a control test. Here cell-free exudate is used within the pipet. The leukocytes exhibit a random motion, typical of indifference to a test substance.

It was evident in early tests on normal saliva that any objective method of measuring the chemotactic power of saliva should be based upon statistical analysis. Qualitative methods for evaluating the chemotactic power failed to indicate the dependability of measurements and the significance of variations. In a microscopic field containing no test substance the probability of a cell moving toward or away from an imaginary point is one-half. To test the validity of this statement with regard to standard test conditions in these studies, 115 cells were observed when no attracting substance was present. Four points situated in the 4 quadrants of the fields respectively were

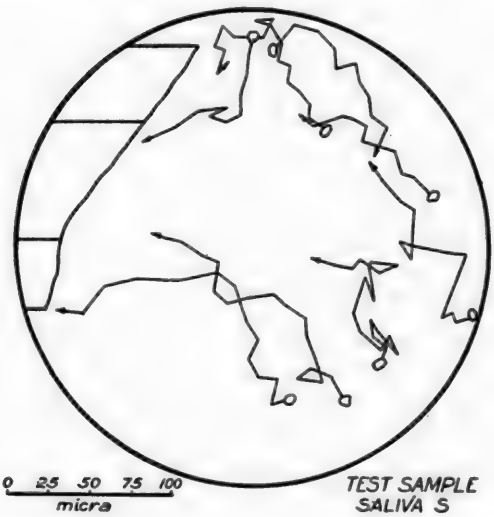

FIG. 1. Camera lucida drawing of movement of leukocytes in microscopic field

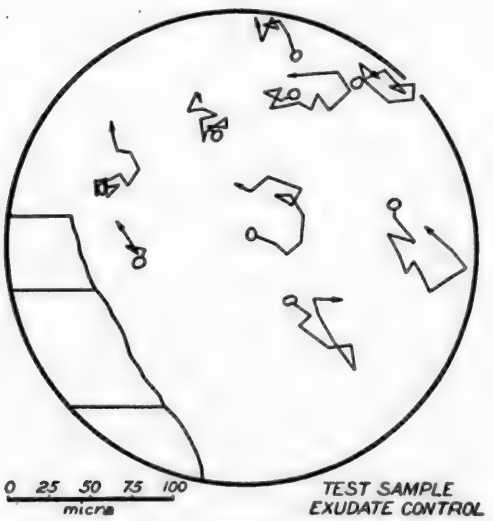

FIG. 2. Random movement of leukocytes in control field

chosen as imaginary sources of attraction. The following table records the per cent of cells attracted toward each of the 4 points.

Points	1	2	3	4
Per cent pos.	50	49	52	46

From these results the conclusion seems justified that when a pipet containing a test substance is inserted into a system such as mentioned

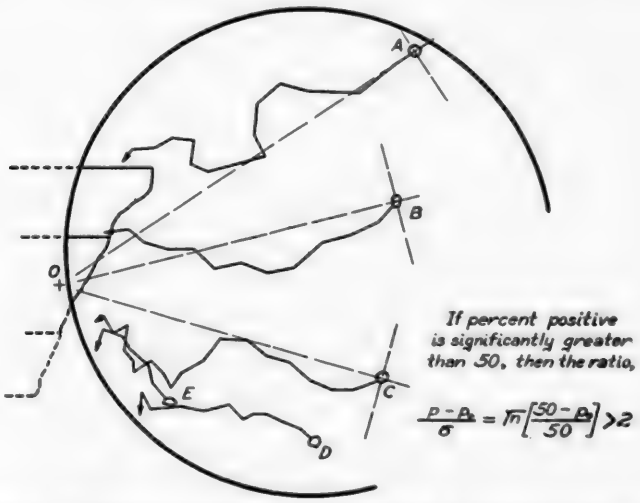

FIG. 3. Application of method of statistical analysis to leukocyte movement

above any preferential movement of the leukocyte is attributable to the pipet, the test substance or a combination of both. *Fig. 3* demonstrates the application of the method of statistical analysis to leukocyte movement. Points A, B, C *etc.*, represent initial positions of leukocytes under observation. Construction lines are drawn from point O, the arbitrarily chosen point source within the pipet, to points A, B and C respectively. At point A a perpendicular is erected to AO dividing the field for the leukocyte at A into 2 regions, 1 of which contains the pipet. If a cell moved into the region containing the pipet it was said to be positive and if it moved into the region not

containing the pipet it was classed as not being attracted. The same procedure can be carried out for points B, C *etc.* The expression for determining the significance of the measurements is included in the chart.

To determine if the observed per cent of attracted leukocytes is significantly different from 50 per cent, this ratio, i.e.,

$$\frac{p - p_0}{\sigma} = \frac{50 - p_0}{(50/\sqrt{n})} = \sqrt{n}\left[\frac{50 - p_0}{50}\right]$$

is calculated, where p = the expected per cent of attracted leukocytes (= 50 per cent), p_o = the observed per cent of attracted leukocytes, n = the number of leukocytes observed and $\sigma = \sqrt{\frac{pq}{n}} = \sqrt{\frac{50 \times 50}{n}} = \frac{50}{\sqrt{n}}$

If the ratio is: (a) Less than 2, the observed per cent of attracted leukocytes is insignificantly different from the expected 50 per cent and the motion of the leukocytes may be considered random. (b) Greater than 2, the observed per cent of attracted leukocytes is significantly different from the expected 50 per cent and the motion of the leukocytes may not be considered as random.

Results of the experiments performed on untreated (untr.) saliva are shown in *fig. 4* (taken from Table I.) With each subject the per cent of cells attracted to the saliva exceeded the per cent attracted to the control (cntl.) substance, leukocyte-free exudate. Statistical analysis has shown that this difference between the salivas and the control is significant. No attempt has been made to date to interpret individual variations.

Since saliva is a complex mixture, the question arises as to what factors in saliva are responsible for the attraction of leukocytes. To answer this question in part, an attempt has been made to remove a few of the components. Centrifugation at 3000 r.p.m. for 10 minutes has eliminated some of the bacteria, debris and salivary corpuscles. All bacteria were "inactivated" in the saliva of subject S by heating to 56°C. for 30 minutes. The salivas of subjects A and D were heated to 70°C. for 30 minutes. The data thus obtained were included in our calculations only if an agar slant inoculated with a

sample of the saliva immediately after heating, remained steril for 48 hours at 37°C. The salivas of subjects D and A were filtered through

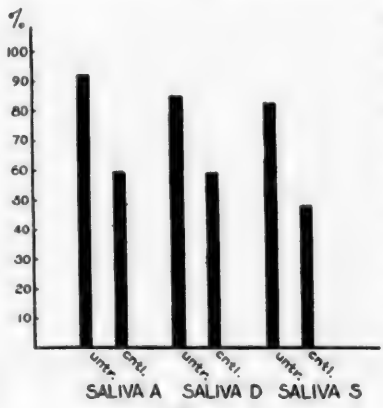

FIG. 4

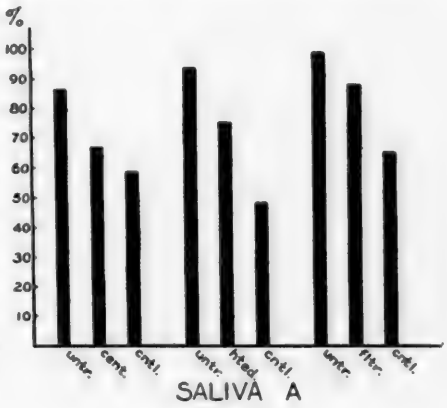

FIG. 5

FIG. 4. Results of experiments on untreated salivas
FIG. 5. Effects of centrifugation, heating and filtration (Saliva A)

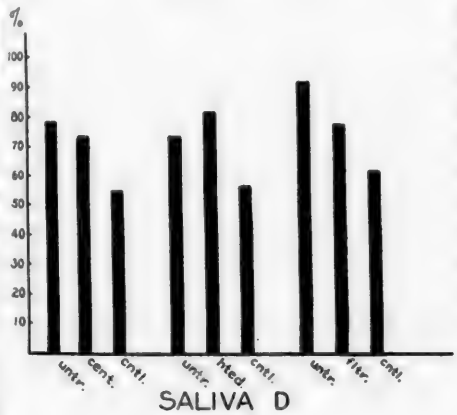

FIG. 6

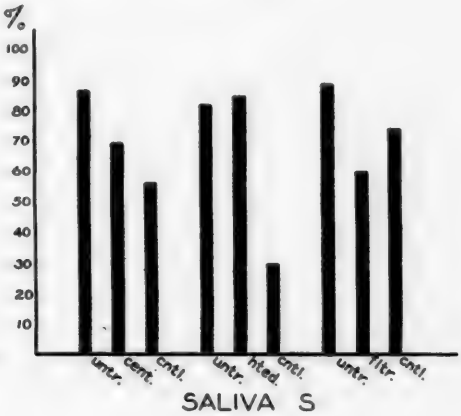

FIG. 7

FIG. 6. Effects of centrifugation, heating and filtration (Saliva D)
FIG. 7. Effects of centrifugation, heating and filtration (Saliva S)

a Jena G5/3fritted glass filter so that only filtrable components remained as test material.

TABLE I

Results of observations on salivas A, D and S

	UNTREATED		CENTRIFUGED		HEATED		FILTERED		CONTROL	
	+	−	+	−	+	−	+	−	+	−
Saliva A										
Cells	147	12							86	61
Per cent positive	92 ± 3.3								59 ± 6.1	
Cells	34	5	24	12					26	19
Per cent positive	87 ± 2.1		67 ± 2.8						58 ± 3.3	
Cells	37	3			30	10			21	23
Per cent positive	93 ± 1.7				75 ± 2.7				48 ± 3.3	
Cells	43	1					28	4	20	11
Per cent positive	98 ± 1.0						88 ± 1.9		65 ± 2.7	
Saliva D										
Cells	121	23							77	53
Per cent positive	84 ± 4.4								54 ± 5.6	
Cells	28	8	27	10					20	17
Per cent positive	78 ± 2.5		73 ± 2.7						54 ± 3.0	
Cells	19	7			28	6			14	11
Per cent positive	73 ± 2.3				82 ± 2.2				56 ± 2.5	
Cells	35	3					23	7	20	13
Per cent positive	92 ± 2.8						77 ± 2.3		61 ± 2.8	
Saliva S										
Cells	62	14							35	38
Per cent positive	82 ± 3.4								48 ± 4.5	
Cells	27	4	18	8					18	14
Per cent positive	87 ± 1.9		69 ± 2.4						56 ± 2.8	
Cells	23	5			23	4			5	19
Per cent positive	82 ± 2.0				85 ± 1.5				21 ± 1.7	
Cells	17	2					18	12	19	7
Per cent positive	89 ± 1.3						60 ± 2.7		73 ± 2.3	

The effects of the above alterations (*i.e.* centrifugation and filtration) brought about, in general, a diminution but not elimination of positive chemotaxis. This is demonstrated in *figs. 5, 6, 7* and Table I. Filtration of saliva S apparently removed the leukocyte-attracting principle of this saliva (*fig. 7* and Table I). It will be observed from *figs. 6 and 7* that the heated saliva appeared to possess a stronger chemotactic influence than did the untreated saliva of parallel observations. Heating reduced the chemotactic activity of saliva A (*fig. 5*).

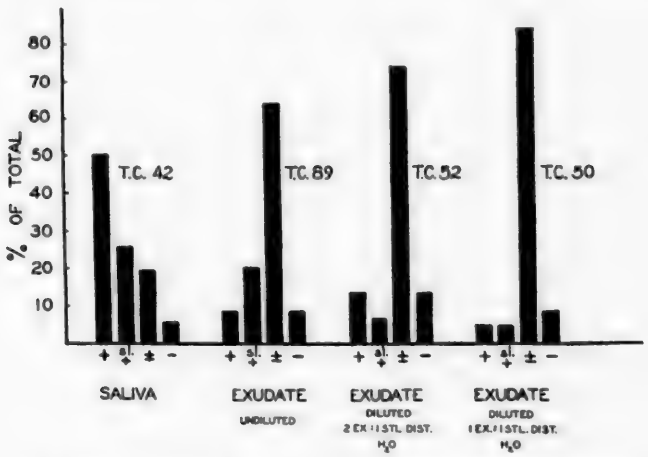

FIG. 8. Effect of diluting exudate T. C. = total number cells observed.

Table I expresses the results of the observations made on the salivas of subjects A, D and S; here the total number of cells studied is given for comparison with the per cent of cells attracted. The error is stated in terms of the standard deviation.

Since saliva is hypotonic to blood serum, the question arose as to whether differences in tonicity between the substance in the pipet and the exudate outside the pipet could be partly responsible for attraction. With this in mind, the tonicity of the material in the pipet was changed by diluting the exudate in varying degrees with steril distilled water. *Fig. 8* shows the results of these tests. The height of the columns represents the percent of cells studied which can be

classed as positive, slightly positive, indifferent and negative. These tests were evaluated by an earlier method. The results indicate that within the limits studied differences in osmotic pressure do not affect the per cent of cells attracted. This finding eliminates the possibility that the attracting effect of saliva is due to a difference in osmotic pressures between saliva and exudate.

CONCLUSIONS

Observations made on the salivas of subjects A, D and S by an *in vitro* method, show that in each case the per cent of leukocytes attracted to the saliva was significantly greater than the per cent attracted to the control substance. When the saliva of each individual was altered by centrifugation, this chemotactic property was not destroyed. Untreated saliva possesses a greater attraction for leukocytes than either centrifuged or filtered saliva. In 2 cases (D and S) the per cent of cells attracted to the heated saliva was insignificantly different than the per cent attracted to unheated saliva; in 1 case (A) the per cent positive with heated was less than with unheated saliva.

Differences between the tonicity of the material within the pipet and the tonicity of the leukocyte-containing exudate were found to exert no appreciable attractive or repulsive influence upon the leukocytes when the tonicity of the test substance was varied to cover the range of normal saliva.

The author wishes to express her appreciation to Mr. Morton Kramer, Department of Biostatistics, Johns Hopkins University for the development of the statistical method involved, and to Doctor J. L. T. Appleton for his support and interest in this work.

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REACTION OF THE PULP TO CALCIUM HYDROXIDE[1]

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Vital pulp amputation goes back as far as the work of Codman (1851). Later Cutler, Sangster, Hopewell-Smith and Davis pioneered in this work. Among the German workers the vital pulp amputation method has been given much attention in the past 10 years. Neuwirth, Hellner, Feldmann, Munch and Herrmann have investigated this problem recently. Hermann produced a drug of $Ca(OH)_2$ in combination with other salts (*Calxyl*). Many favorable reports have been published concerning uses of this drug. Other drugs and plain dentin dust have been claimed to have produced similar good results (Feldmann, Hellner and others).

The inorganic content of dentin is primarily a hydrated tricalcium phosphate and as such should follow the laws of mass action in relation to the solubility product. The blood is normally saturated or supersaturated with Ca and PO_4 ions, and hence any increase in Ca or PO_4 ions would cause a precipitation or laying down of calcium salts. A material which contains either Ca or PO_4 in a combination which would be easily ionized when brought in contact with the surface of the pulp should react in this manner. This probably is the action of $Ca(OH)_2$. The normal mechanism of deposition of bone salts has been shown to depend to a large extent on an increase of PO_4 ions due to the liberation of inorganic phosphate from blood or tissue by means of a phosphatase enzyme. As bone phosphatase is known to act best in an alkaline medium and as the solubility product decreases with increased alkalinity the conditions here approach an optimum. $Ca(OH)_2$ has a pH of 12.4. Under these conditions there is a high pH and a large abundance of calcium ions and organic mate-

[1] Presented at the 17th General Meeting of the International Association for Dental Research, Cleveland, Ohio, March 18–19, 1939.

rial as a matrix. For this reason a rapid precipitation of calcium phosphate might be expected.

For the formation of normal dentin the organic collagenous fibrous matrix in which the calcium salts become deposited is first required;

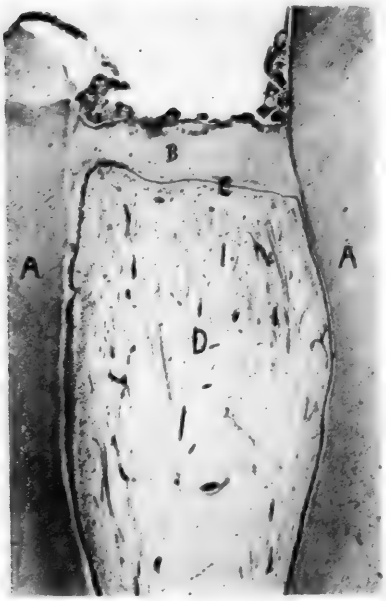

FIG. 1

FIG. 2

FIG. 1. Histological specimen of tooth. A—Dentin wall, B—Dentin bridge, C—Odontoblasts, D—Pulp.

FIG. 2. A higher magnification of a part of the dentin bridge (B) as shown in *fig. 1*. A—Dentin wall, B—Dentin bridge, C—Odontoblasts underneath dentin bridge, C_1—Odontoblasts at dentin wall, D—Pulp, E—Zone of amorphous calcification with cell enclosures.

the protoplasmic processes of the odontoblasts must be included in the dentin matrix if it is to be considered normal dentin.

For pulp amputations two essentially different methods have been employed. In one the pulp has been devitalized, chiefly with arsenic, and the remaining pulp tissue mummified by formaldehyde and creosote or some of their derivatives. By the other method a part of the pulp is cut off and the rest is left alive in the root canal. It

is the aim of this method to cause the pulp to produce dentin on the surface of the cut wound and to wall off the pulp canals from the cavity.

This investigation was started with *Calxyl*, but it was soon found out that plain $Ca(OH)_2$ produced the same results.

FIG. 3. Another area of the same tooth as in *figs. 1* and *2*. A—Pulp, B—Dentin bridge, C—Area of accumulation of inflammatory cells, D—Dentin wall, E—Dentin formation around piece of dentin dust.

METHOD

Surgical cleanliness is obtained and maintained throughout the operation by applying the rubber dam and sterilizing the field with 3 per cent tincture of iodine or 70 per cent alcohol. Local anesthesia is used. Good access to the pulp chamber is essential, all caries being removed before the removal of the roof of the pulp chamber. The bulbous portion of the pulp is then severed from the root portion with sharp spoons. Bleeding is controlled by the application of sterile cotton pellets soaked in a saturated solution of calcium hydroxide. $Ca(OH)_2$ in the form of a thick creamy paste is used to cover the severed ends of the root pulps and, in order to avoid pressure and deleterious affects from the cement on the pulp, a thin coat of paraffin is flowed over the $Ca(OH)_2$. This paraffin is covered with cement. A final restoration for the posterior teeth is made with some

metal filling, the cavity being prepared so that the metal does not rest entirely upon the cement base but on some sound dentin.

150 cases have been treated by the described method during the last 2 years. *Calxyl* was used on 60 cases, $Ca(OH)_2$ on 90 cases. The radiographic checkup revealed no apical changes in 71 per cent of the cases.

CASE REPORT

A 9 year old boy presented with large cavities in his anterior teeth. The decay has penetrated to the pulp and, as the apex was still open, a vital amputation was performed using $Ca(OH)_2$. After 8 months the crown of the tooth broke off because of a minor accident, and it was necessary to extract the tooth. The radiograph showed that the crown had been lost and a bridge covering the amputated pulp surface. No changes could be observed in the periapical region. The histologic specimen revealed a dentin bridge covering the amputated pulp for the entire width of the pulp canal (*fig. 1.*). The odontoblastic layer was continuous below the newly formed dentin bridge. The odontoblasts are somewhat shorter beneath the dentinal bridge than along the normal pulpal wall but the dentinal tubuli are quite regular and well arranged and the dentin shows an uncalcified layer toward the odontoblasts as it does under normal conditions (*fig. 2.*). The pulp shows capillaries and nerve fibers which seem to end just below the dentin bridge and differs from the normal only by the presence of a large number of wandering cells. While wandering cells are normal constituents of the human pulp they are in excess in this specimen and show typical characteristics of Maximow's *polyblasts*.

In study of serial sections these wandering cells were found forming what we may term a chronic abscess although some of the characteristics of an abscess are missing. In *fig. 3* the cell collections are lymphocytes, plasma cells or polyblasts, no leucocytes being found. This indicates that the inflammatory reaction is quite chronic. We see no reason to believe that this inflammatory reaction is of recent origin but rather think that it is the remnant of the irritation of 8 months ago.[2] In the vicinity of this cell collection we can see a large number of resting wandering cells, which would also indicate that the process is very inactive and decreasing rather than increasing. Further away from the dentin bridge the pulp shows the most normal appearance.

DISCUSSION

This method has also been tried in deciduous teeth. *Fig. 4* shows a similarly favorable result at the end of a $3\frac{1}{2}$ months' period. The pulp tissue shows no signs of inflammation and the dentin bridge completely covers the pulp surface. Odontoblasts line the dentin with their processes extending into the dentin.

In almost every case of vital pulp amputation a zone of structureless dentin is found (*fig. 4*). On the outside of the dentin bridge there is a dark, amorphous, structureless layer showing at some places cell enclosures or empty spaces. This is probably the result of the first chemical reaction between the calcium hydroxide and the tissue surface

[2] W. Bloom (Univ. of Chicago) confirmed our belief in viewing the specimen.

of clotted blood and degenerated injured pulp cells. Into this matrix, most probably, the first calcium phosphate precipitation takes place giving the proper base for true dentin formation. From our observations we believe that dentin formation takes place not only in a healthy pulp but also in the presence of inflammatory reactions. The inflammatory cells may later disappear and healthy pulp tissue take their place or the reaction may follow a quite different course. After the pulp amputation an indifferent calcified layer is deposited; subsequently and continuous with this a definite regular dentin bridge is

FIG. 4. Dentin bridge in a deciduous tooth. A—Pulp, B—Dentin bridge, C—Odontoblasts, D—Pulp.

formed. The inflammatory reaction which may be present as a result of the exposure, or the mechanical or chemical irritation, may not stop but will progress until the pulp completely degenerates. *Fig. 5* is from a case with this outcome, a lower molar on which a pulpotomy was performed 7 months before extraction of the tooth. The dentin bridge was strikingly well developed but the pulp entirely disintegrated. Only a few necrotic cell nuclei and necrotic tissue remnants were found. Near the apex an acute inflammatory reaction was seen with a large number of leucocytes present. The outside of the dentin bridge is characteristically amorphous.

This finding, which is not the only failure in our experiment, strongly indicates that in performing this vital pulp amputation by this method, a number of unsolved problems must be faced. It has not, as yet, been determined why one case should turn out favorably and successfully and another fail. It is not known whether it was the condition of the pulp at the time pulpotomy was performed, the technique employed or the difference in the vital reaction of the individual.

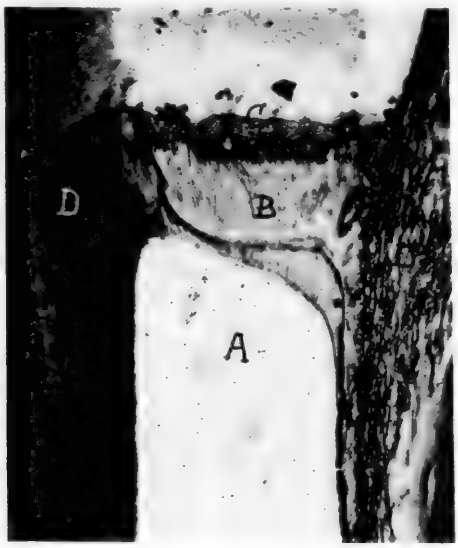

FIG. 5. Dentin bridge with subsequent complete pulp degeneration. A—Pulp, B—Dentin bridge, C—Area of amorphous calcification, D—Dentin wall.

SUMMARY AND CONCLUSIONS

1. The action of $Ca(OH)_2$ and *Calxyl* on the pulp tissue has been studied. The formation of an amorphous calcified layer on the cut pulp surface has been observed. This layer of amorphous calcium formed the matrix for the regular dentin formation, resulting in a bridge covering the exposed pulp tissue.

2. From a practical view point this method holds possibilities as an emergency treatment in cases where other methods are quite

likely to fail. These cases are teeth in which either the root end is not completely formed or, as in deciduous teeth, resorption is taking place.

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PHYSICAL PROPERTIES OF SOME OF THE NEWER DENTURE PLASTICS[1]

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There is probably no field in dentistry in which changes in type of materials have been so rapid during recent years as in prosthesis in connection with denture base materials. The search for a substitute for vulcanite, which would exhibit better esthetics and yet be equally or more efficient, has been long and wide. The principal efforts in this direction have been concentrated mainly in the synthetic resin field. Although physical tests of these materials do not tell the entire story as to their probable success or failure in clinical practice correlations in this regard have been successful in most cases. For example, had the prosthetist paid more attention to the fatigue tests of Barber (1) he might not have been so optimistic of the vinyl resins for use as denture base materials.

Other outstanding papers on the physical properties of these materials are those of Kimball (2), and Sweeney and Schoonover (3). The last named paper reported on flexual fatigue and tensile properties, impact strength, water sorption, thermal conductivity, transverse bend, color life, and the chemical composition of vulcanite.

Since these researches have been published, other materials have appeared on the market. The present research is concerned with the testing of 6 of these newer materials. A dental vulcanite was also included for purposes of comparison. The materials studied are given in Table I. (Not listed in order of code letters in subsequent tables.)

All products were purchased upon the open market, with the exception of the methyl-methacrylate resins, which were not available for purchase at the time the investigation started.[2]

[1] Presented at the 17th General Meeting of the International Association for Dental Research, Cleveland, Ohio, March 19, 1939 (*J. D. Res.*, 18: 248, 1939).

[2] The authors are indebted to Messrs. Kerr and Vernon for supplying these materials gratis until they were placed on the market.

TESTING PROCEDURE AND RESULTS

The materials were tested for tensile properties, impact strength, and water sorption (increase in weight and length). A few experiments were done on color life. The testing procedures described by Sweeney and Schoonover (3), or Barber (1) were employed in most cases. However, instead of using aluminum molds, all specimens were treated in a special flask, exactly as directed by the manufacturer. At least 5 specimens were tested each time, with the exception of the water sorption determination.

TABLE I

List of materials studied

NAME	MANUFACTURER
Crystolex	Detroit Dental Manufacturing Company
Densene	Cosmos Dental Products, Inc.
Duratone	Iteco Dental Manufacturing Company
Exelon	Exelon Manufacturing Division
Luxene 37	Bakelite Dental Products, Inc.
Vernonite	Vernon-Benshoff Company

TABLE II

Impact strength

CODE LETTER	MATERIAL	NO. OF SPECIMENS	AVERAGE IMPACT STRENGTH (CM.-KG./CM^2.)
A	Methyl-methacrylate resin	9	9.6 ± 1.2
A′	Methyl-methacrylate resin (transparent)	5	13.0 ± 1.1
B	Methyl-methacrylate resin	5	11.4 ± 1.0
C	Acrylic-vinyl resin*	5	18.4 ± 1.2
D	Phenol-formaldehyde resin	5	7.6 ± 0.6
E	Phenol-formaldehyde resin	7	3.4 ± 0.8
F	Phenol-formaldehyde resin	7	3.4 ± 0.7
G	Olive base rubber	5	23.4 ± 4.1

* Type of compound not known.

Impact. Unmatched specimens of 2.5 x 10 x 70 mm. dimensions were employed. An Amsler Impact Machine (Charpy type) of 10 cm.-kg. capacity was used. The distance between the mounting supports was 28.5 mm. in all cases. The results are given in Table II. A′ is a transparent form of A; with the exception of A′ and G, all of the materials were gum pink in color.

Tensile Tests. In order to simplify the machining of the specimen with the tools available, straight specimens were used, 4 x 6 x 140 mm. in dimension, for all the materials

except the phenol-formaldehyde resins, the dimensions of which were 4 x 7 x 140 mm. in order to give strength during the machining process.

A 600-pound capacity Emery testing machine, screw action, hydraulic recording, and hand operated, was employed. Ten-pound incremental loads were used, which were applied every 20 seconds at room temperature. The strain was measured with a Tinius Olsen averaging extensometer, 2-inch gage length. The results shown in Table III are the averages for at least five specimens. Typical stress-strain diagrams are presented in the *fig. 1.*

The proportional limit was determined as the stress where a loading increment produced 1.25 as much strain as was produced by the same increment at lower stresses (4). The method of Barber (5) was followed for the determination of the modulus of elasticity, i.e., by using the portion of the stress-strain curve from approximately 1200 pounds per

TABLE III

Tensile properties

CODE LETTER	PROPORTIONAL LIMIT		MODULUS OF ELASTICITY		MODULUS OF RESILIENCE		ULTIMATE TENSILE STRENGTH		SET 50 MM. GAGE LENGTH
	(Lb./In.²)	(Kg./cm.²)	(Lb./In.²)	(Kg./cm.²)	(In. Lb./In.³)	(cm.-kg./cm.³)	(Lb./In.²)	(Kg./cm.²)	Percent
A	3900 ± 100	275	330,000 ± 0	23,200	23	.8	6900 ± 100	486	0.1
A′	3900 ± 100	275	360,000 ± 10,000	25,400	21	.8	7900 ± 200	557	0.2
B	3800 ± 100	268	340,000 ± 10,000	24,000	21	.8	7300 ± 200	514	0.2
C	4200 ± 300	296	580,000 ± 10,000	40,900	15	.6	7700 ± 100	543	7.4
D	5600 ± 300	314	520,000 ± 10,000	36,700	30	1.1	5600 ± 300	395	
E	2200 ± 200	155	500,000 ± 30,000	35,200	5	.2	2200 ± 200	155	
F	2800 ± 400	198	380,000 ± 10,000	26,800	11	.4	2800 ± 400	197	
G	3900 ± 100	275	350,000 ± 20,000	24,700	22	.8	7900 ± 100	557	0.7

square inch to within approximately 500 pounds per square inch below the established proportional limit. The modulus of resilience was computed in the usual manner. The permanent set was determined in the same manner as the percentage elongation for a metal. A 50-mm. gage length was employed. No permanent set could be measured for the phenol-formaldehyde resins.

Water Sorption. Unfortunately, only 1 trial for each material was made for this test. Measured samples of each material, except vulcanite, were weighed and placed in a desiccator for 2½ days, and then re-weighed. They were then placed in distilled water at room temperature. Periodically, the excess water was carefully wiped off the specimen, and the gain in weight determined in comparison with the weight after desiccation.

Another set of specimens which had been specially prepared for accurate length

measurement by a micrometer caliper were similarly treated, and the percentage increase in length computed. The results of the water sorption tests are given in Table IV.

Color Life. Specimens of resins, with the exception of E, were exposed to sunlight for varying periods of time (Table V). During the exposure, one-half of the specimen was covered with tin foil.

TABLE IV

Sorption of water at room temperature

CODE LETTER	DECREASE IN WT. AFTER DESSICATION	2 DAYS	5 DAYS	9 DAYS	15 DAYS	22 DAYS	33 DAYS	44 DAYS	INCREASE IN LENGTH AFTER 44 DAYS
	(Mg/Cm²)	(Mg/Cm²)	(Mg/Cm²)	(Mg/Cm²)	(Mg/Cm²)	(Mg/Cm²)	(Mg/Cm²)	(Mg/Cm²)	(Per Cent)
A	0.02	0.06	0.11	0.15	0.17	0.18	0.20	0.21	0.43
A′	0.02	0.07	0.10	0.14	0.18	0.19	0.21	0.21	0.43
B	0.02	0.07	0.10	0.14	0.18	0.20	0.21	0.22	0.42
C	0.01	0.02	0.03	0.04	0.05	0.06	0.06	0.07	0.13
D	0.02	0.02	0.04	0.05	0.06	0.06	0.08	0.08	0.17
E	0.04	0.03	0.04	0.07	0.09	0.09	0.11	—	0.55
F	0.02	0.03	0.04	0.06	0.08	0.09	0.12	0.13	0.23

TABLE V

Tests for color life

CODE LETTER	EXPOSURE TO SUNLIGHT (HOURS)	EFFECT
A	340	Slightly bleached on surface
A′	340	None
B	340	None
C	30	None
D	340	Definite brown
F	116	Light brownish yellow

COMMENT

It is doubtful whether or not much importance can be attached to the tensile properties concerned with elasticity. As may be noted from *fig. 1*, the stress-strain curves are not as definite as similar curves for metals. These materials are definitely plastics, and their plasticity is evident even though a constant time loading was employed. How-

ever, the data show differences in the measurements, which may be significant, whatever the figures may represent physically.

The methyl-methacrylate resins appear to be equal to vulcanite so far as tensile properties are concerned (Table III), except in their capacity to withstand sudden stress without fracture. This latter

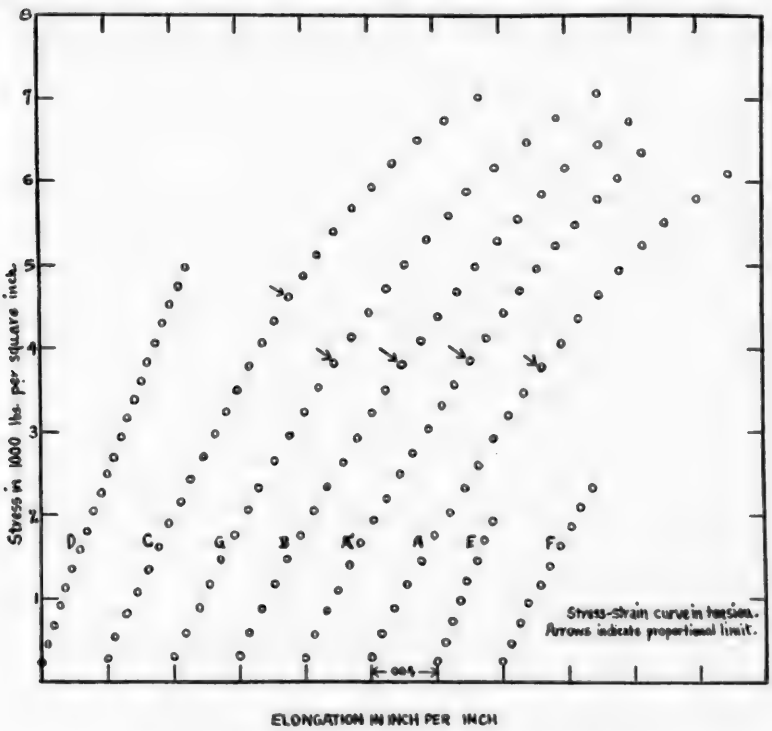

FIG. 1. Typical stress-strain diagrams for materials tested

property is shown by a comparison of their set (Table III) and impact strength (Table II). However, if it is assumed that the modulus of resilience is a measure of the resistance to stress from impact force within the proportional limit, the methyl-methacrylate resins should be as good as vulcanite in this respect. All 3 of the methyl-methacrylate resins appear to be equal in every respect, so far as all their

physical properties are concerned, within the limits of experimental error.

In the case of resin C, it is doubtful whether the measurement of permanent set should be taken as a criterion of resistance to breakage by a mechanical shock. The permanent set for this resin is over 1000 per cent greater than that for vulcanite, yet its impact strength is only 78 per cent of that of vulcanite. Although its modulus of elasticity is higher than all the other materials, its modulus of resilience is comparatively low. Furthermore, it was found that a strip of the material could be bent double on itself at room temperature without rupture.

Phenol-formaldehyde resin D is outstanding among the materials of its classification, so far as tensile and impact properties are concerned. This resin is presumably manufactured by the same firm as the phenol-formaldehyde resin tested by Sweeney and Schoonover,[3] but it is supposedly an improvement over the material tested by them. Sweeney and Schoonover (6) give 443 kilograms per square centimeter, 0.2 per cent, and 3 centimeter-kilograms per square centimeter for ultimate tensile strength, set, and impact strength, respectively. As may be noted from Table III, this newer material is somewhat weaker in tensile strength, but it is stronger in impact strength (Table II). However, the difference is not great in either case. The other 2 phenol-formaldehyde resins are definitely inferior to resin D in mechanical properties.

The results for the vulcanite material are in satisfactory agreement with the data of Sweeney and Schoonover (6). Also, the tensile data for resin E are in agreement with Kimball (2).

So far as the tests for water sorption are concerned, (Table IV) the methyl-methacrylate resins on the average imbibed approximately 60 per cent more water in 44 days than resin D for example, and increased in length approximately the same amount over this resin. It is not known whether or not warpage of the specimens were a factor in these latter tests. Furthermore, the clinical application of water sorption tests in general is not clear at the present time.

[3] "Presumably," because it is against the policy of the American Dental Association Research Commission to give out the trade names of the products used in their investigations.

The tests for color life (Table V) are not entirely conclusive because of the variable times of exposure employed. It is certain that 30 hours of exposure is not sufficient to draw any conclusions for resin C. Furthermore, had resin F been exposed as long as resin D, it might have become as badly discolored as D. However, it can be stated that the color life of the phenol-formaldehyde resins tested is not as good as that of the methyl-methacrylate resins, so far as exposure to sunlight is concerned.

SUMMARY AND CONCLUSIONS

Three methyl-methacrylate, 1 acrylic-vinyl, and 3 phenol-formaldehyde resin denture base materials have been studied in connection with certain physical properties. So far as these materials are concerned, it may be concluded from the data presented that: 1. In comparison with vulcanite, the methyl-methacrylate resins are nearly equal in every respect, so far as their tensile properties are concerned. The same may be said of the acrylic-vinyl resin, except that its permanent set is much higher. Two of the phenol-formaldehyde resins are inferior to vulcanite, but the third is less flexible, more resilient, weaker under tensile stress, and more brittle than vulcanite. 2. Vulcanite is definitely superior in impact strength to all the materials, with the acrylic-vinyl resin second. The methyl-methacrylate resins are approximately one-half as strong in this regard, and the phenol-formaldehyde resins are definitely weak in comparison. 3. After 44 days of immersion, the methyl-methacrylate resins take up approximately 60 per cent more water than the acrylic-vinyl resin and one of the phenol-formaldehyde resins. However, the clinical significance of these tests is not clear. 4. The color life of the methyl-methacrylate resins, as tested by exposure to sunlight, is much better than that of the phenol-formaldehyde resins. No conclusion can be drawn as to the color fastness of the acrylic-vinyl resin.

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(1) Barber, R., *J. A. D. A.*, **21**: 1969, 1934.
(2) Kimball, H. D., *J. A. D. A.*, **23**: 579, 1936; *Ibid.*, **23**: 1904, 1936.
(3) Sweeney, W. T., and Schoonover, I. C., *J. A. D. A.*, **23**: 1498, 1936.
(4) *Idem, ibid.*, p. 1501.
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(6) Ref. 3., p. 1500.

THE EFFECT OF HARD AND SOFT DIETS ON THE GINGIVAL TISSUES OF DOGS[1]

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Vague references to the effect of hard and soft foods on oral health may be found in the literature. It has been suggested that the absence of periodontal pockets in Eskimos is due to the fact that their native diets are very coarse and difficult to masticate and, on the other hand, it has been claimed that periodontal pockets may be demonstrated in people living on hard diets. In general, consistency of foods is considered a contributing factor to periodontal disturbances.

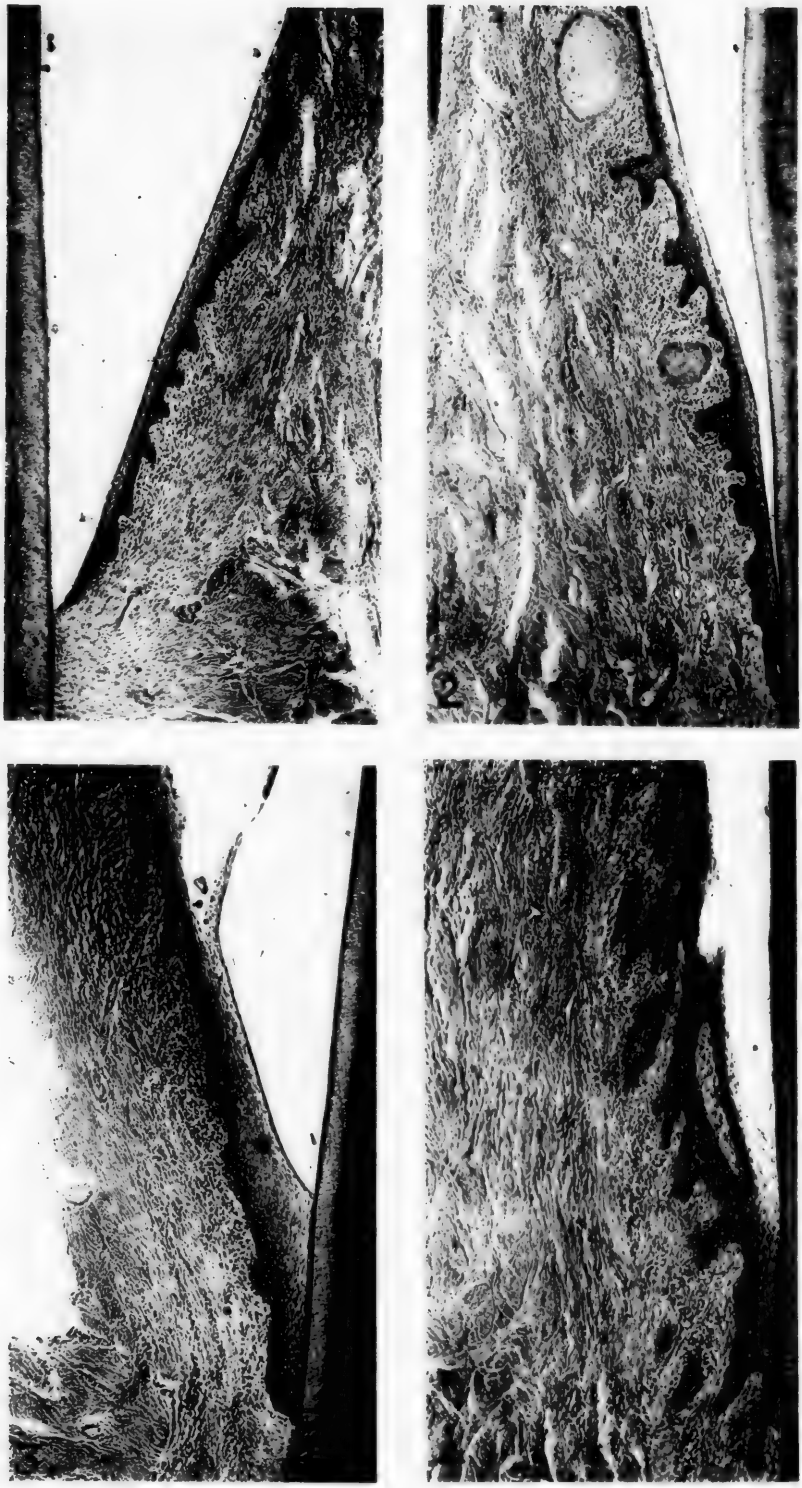
Clinically, it has long been observed that individuals living on a soft food regimen have so-called "soft" gingivae, while those living on coarse food have "hard" gingivae. In the one case the gingival tissues may be soft, dark red, and bleed easily; in the other they are firmer, pink in color and more resistant to injury. There has been little effort to verify these observations by experiment. The object of this experiment was to verify or refute this clinical observation by subjecting dogs to the influence of hard and soft foods.

METHOD

Two groups of young dogs were placed on diets, identical chemically but different physically. The control group, received its rations in the form of dog biscuits of hard consistency. The experimental group received the same food ground to a powder and mixed with water to form a mash. The basic food was dog chow (Purina), an adequate nutriment consisting of meat, wheat germ oil, dried skim milk, cod liver oil, barley malt, molasses, oat and corn cereal, wheat bran, and iodized salt. The analysis of this food was: Protein 20 per cent, Fat 3 per cent, Fiber 6 per cent, N-free extract 50 per cent, Carbohydrates 56 per cent, Ash 6 per cent, Caloric Value 1500 calories/lb., Ca/p ratio 1.4/1. Until the animals became accustomed to this diet it was supplemented with milk and hamburger.

The control group consisted of 2 animals, and the experimental group of 4 animals. The experiment was run for 14 months after the animals had been weaned. Three months after the experiment was started, 1 of the controls died of an undetermined cause and was replaced by another animal, so that 1 control was on hard diet for 14 months while

[1] Presented at the 17th General Meeting of the International Association for Dental Research, Cleveland, Ohio, March 18–19, 1939 (*J. D. Res.* **18**: 237, 1939).



FIGS. 1–4

the other was on this diet for 11 months. One of the animals on the soft diet died 6½ months later, and was not replaced.

After the animals were killed their jaws were fixed in formalin. The lower jaws were used for a separate study, and the work here reported was done on the upper jaws. These were decalcified in 2 per cent nitric acid, embedded in celloidin, sectioned at 20–25 micra and stained in Delafield's hematoxylin and alcoholic eosin. The sections shown in this study were all made from the maxillary third incisor teeth.

RESULTS

Macroscopic description of jaws: The jaws of the animals living on the soft diet showed inflammation at the crests of the gingivae. The gingival portions of the crowns of the teeth were covered with plaques, and there was impaction of food under the gingival margins. The gingivae were dark and soft. In the animals on the hard diet, the teeth were comparatively free of debris and plaques. The gingivae were paler and firmer, and did not appear "dirty" as did those on the soft diet.

Microscopic description: Fig. 1 is a photograph of a section taken from an animal on the hard diet. The epithelial layer of the gingival crevice is thin, definite and normal. The stratum germinativum appears normal, with typical short papillary downgrowths. The connective tissue papillae underlying the basement membrane are short and exhibit a slight amount of round-cell infiltration. The deepest part of the epithelial attachment ends in a definite line at the cemento-enamel junction. On the whole, this may be considered a picture of a healthy gingiva. *Fig. 2* is a photograph of a section taken from the other control animal. The epithelium is similar to that in the preceding photograph. The epithelial papillae are short and the basement membrane is well defined, with only a slight amount of subepithelial round-cell infiltration. The epithelium at the deepest portion of the gingival attachment is slightly proliferated but the general appearance is one of a normal gingiva.

FIG. 1. Hard diet. Crevice epithelium and subepithelial tissues normal. Hematoxylin and eosin × 70.

FIG. 2. Hard diet. Slight proliferation of epithelial papillae, section otherwise normal. Hematoxylin and eosin × 70.

FIG. 3. Soft diet. Increased subepithelial infiltration and proliferation of attachment along cementum. Hematoxylin and eosin × 70.

FIG. 4. Soft diet. Extensive papillary downgrowth and subepithelial infiltration with marked proliferation of epithelial attachment along cementum. Hematoxylin and eosin × 70.

Fig. 3 shows a section taken from an animal on the soft diet. The epithelial papillae have definite downward extensions, and there is a material increase in round-cell infiltration. There is also a downward proliferation of the epithelial attachment along the cementum. This section shows a condition which is only slightly abnormal. *Fig. 4*

FIG. 5. Soft diet. Loss of epithelium, dense subepithelial infiltration and extensive papillary downgrowth. Mass of debris impacted in gingival crevice. Hematoxylin and eosin × 70.

shows a gingival area from another animal on the soft diet. There is desquamation of the surface layers of the epithelium and proliferation of the papillary downgrowths, with marked round-cell infiltration which has penetrated through the epithelium. The proliferation of epithelium along the cementum is marked. *Fig. 5* is a photograph

of a section taken from the third animal on the soft diet. The epithelium of the gingival crevice shows marked pathological changes. Toward the gingival crest there is a loss of epithelium and an accumulation of debris, which from its staining reaction appears to be impregnated with calcareous material. There has been extensive proliferation of the epithelium lining the gingival crevice and marked downward extension of the papillae of the stratum germinativum. There is a dense round-cell infiltration throughout the underlying connective tissue, which infiltration has penetrated through the epithelium.

DISCUSSION

This study indicates that gingival changes due to consistency of diet are variable, but that there is a difference between gingivae exposed to hard diets and those exposed to soft diets. Soft diets tend to produce more pathological change. This suggests that the changes might be the result of lack of stimulation, leading to stasis of the gingival circulation and a softening of the tissues. Under these conditions the impaction of the smaller particles of food in a soft diet might occur more readily, leading to tissue irritation and proliferation of the epithelial downgrowths.

Conversely, it is possible that coarse and hard foods would promote tissue stimulation, resulting in firm, turgid gingivae which would cling to the teeth and thus reduce the chances of the impaction of particles below the gingival margins.

CONCLUSIONS

Histological findings confirm the clinical observation that soft diets tend to produce pathological changes in the gingivae.